GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

Founded in 1916
by
GEORGE H. SHULL
with the cooperation of

WILLIAM E. CASTLE
EDWIN G. CONKLIN
CHARLES B. DAVENPORT
BRADLEY M. DAVIS
EDWARD M. EAST
ROLLINS A. EMERSON
HERBERT S. JENNINGS
THOMAS H. MORGAN
RAYMOND PEARL

Volume 39 March, 1954 Number 2

TABLE OF CONTENTS

SCHERR, GEORGE H., MARVIN FISHMAN AND R. H. WEAVER, The mutagenicity of some carcinogenic compounds for *Escherichia coli* 141
BENNETT, J. H., Panmixia with tetrasomic and hexasomic inheritance 150
KRAMER, H. H., ROSEMARIE VEYL AND W. D. HANSON, The association of two genetic linkage groups in barley with one chromosome 159
LEVINTHAL, CYRUS, Recombination in phage T2: its relationship to heterozygosis and growth 169
KALTER, HAROLD, The inheritance of susceptibility to the teratogenic action of cortisone in mice 185
NOVITSKI, E., AND G. BRAVER, An analysis of crossing over within a heterozygous inversion in *Drosophila melanogaster* 197
WALKER, B. E., AND E. R. BOOTHROYD, Chromosome numbers in somatic tissues of mouse and man 210
STEFFENSEN, DALE, AND T. J. ARNASON, Frequency of chromosome aberrations produced by fractional doses of X-rays in Tradescantia 220
HANSON, EARL D., Studies on kappa-like particles in sensitives of *Paramecium aurelia*, variety 4 229
CALDECOTT, RICHARD S., B. H. BEARD AND C. O. GARDNER, Cytogenetic effects of X-ray and thermal neutron irradiation on seeds of barley 240

PUBLISHED BIMONTHLY AT LANCASTER, PENNSYLVANIA,
BY GENETICS, INC.,
GENETICS BLDG., UNIVERSITY OF WISCONSIN, MADISON 6, WISCONSIN

(Date of issue, April 13, 1954)

EDITORIAL BOARD

R. Alexander Brink, *Managing Editor*,
James F. Crow, *Associate Managing Editor*,
University of Wisconsin

George W. Beadle
California Institute of Technology

William E. Castle
University of California

Bradley M. Davis
University of Michigan

L. C. Dunn
Columbia University

John W. Gowen
Iowa State College

Donald F. Jones
Connecticut Agricultural Experiment Station

M. M. Rhoades
University of Illinois

George H. Shull
Princeton University

T. M. Sonneborn
Indiana University (Representative of the Genetics Society of America)

L. J. Stadler
U. S. Dept. Agriculture, University of Missouri

Curt Stern
University of California

Alfred H. Sturtevant
California Institute of Technology

Sewall Wright
University of Chicago

Volume 39 MARCH, 1954 Number 2

GENETICS is a bimonthly journal issued in annual volumes of about 600 pages each.

Subscription, $8.00 net a year for complete volumes (January-November). Foreign postage, 50 cents additional. Single copies, $1.50 each, postpaid.

As available, back volumes may be had at $14.00 each and single issues of previous volumes at $2.50 per copy, postpaid. The Science Press will supply information on request as to volumes and numbers available.

Business Correspondence, including change of address, etc., should be addressed to GENETICS, INC., THE SCIENCE PRESS, BOX 749, Lancaster, Pa.

Remittances should be made payable to GENETICS, INC.

Correspondence concerning editorial matters should be addressed to the EDITORS OF GENETICS, Genetics Building, University of Wisconsin, Madison 6, Wisconsin.

Entered as second-class matter at the postoffice at Lancaster, Pa., June 14, 1951, under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in the Act of February 28, 1925, paragraph 4, section 412, P.L.&R., authorized January 9, 1932.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

THE MUTAGENICITY OF SOME CARCINOGENIC COMPOUNDS FOR ESCHERICHIA COLI[1]

GEORGE H. SCHERR,[2] MARVIN FISHMAN[3] AND R. H. WEAVER
Department of Bacteriology, The University of Kentucky, Lexington, Kentucky

Received June 5, 1953

OF the many substances that have been found to be mutagenic several are also carcinogenic and considerable evidence has been accumulating to indicate a relationship between these two properties.

Nitrogen and sulfur mustards were shown by AUERBACH and ROBSON (1944) to be mutagenic for Drosophila, a finding which has been confirmed by DEMEREC (1947a,b), BURDETTE (1952) and others. Nitrogen mustard was found to be mutagenic for Neurospora by BONNER (1946) and for *Escherichia coli* by TATUM (1946) and BRYSON (1948). Sulfur mustard is also mutagenic for Neurospora as demonstrated by HOROWITZ, HOULAHAN, HUNGATE and WRIGHT (1946). That the mustards are also carcinogenic has been shown by BOYLAND and HORNING (1949), HESTON (1949, 1950), and GRIFFIN, BRANDT and TATUM (1951).

Other carcinogens that have been found to be mutagenic include: 20-methylcholanthrene-endosuccinic acid, shown to be mutagenic for Neurospora by TATUM (1947); and chrysene, shown to be mutagenic for yeast by MITRA and SUBRAMANIAM (1950).

LATARJET (1948) and LATARJET, ELIAS and BUU-HOI (1949) reported the mutagenicity of the water-soluble carcinogen 1,2,5,6-dibenzanthracene-α,β-endosuccinate for *E. coli*. The results of the latter study suggested a correlation between carcinogenicity and mutagenicity in that ethyl carbamate was highly carcinogenic and highly mutagenic, while propyl carbamate and isopropyl carbamate were less strongly carcinogenic and less strongly mutagenic, and butyl carbamate was neither carcinogenic nor mutagenic. BARRATT and TATUM (1951), using Tween 80 as a solvent, reported methylcholanthrene, 9,10-dimethyl-1,2-benzanthracene, and 1,2,5,6-dibenzanthracene to increase the mutation frequency in Neurospora 4-fold, m′-methyl-*p*-dimethylaminoazobenzene, and 4′-amino-2,3-azotoluene to increase it 3-fold, and 4-dimethylaminostilbene and acetylaminofluorene to be weaker mutagens or to be inactive. They found all these substances to be much less active than nitrogen mustard and radiations. LATARJET (1948) had been unable to demonstrate mutagenicity of methylcholanthrene for *E. coli*. MAISIN (1951) and MAISIN, LAMBERT and

[1] A portion of this report was presented before the 49th national meeting of the Society of American Bacteriologists (SCHERR and WEAVER 1949).

[2] Present address: Department of Microbiology, The Creighton University School of Medicine, Omaha, Nebraska.

[3] Present address: Department of Microbiology, The Washington University School of Medicine, St. Louis, Missouri.

VAN DUYSE (1951) have summarized the work of MAISIN and his coworkers over a period of several years in which the action of styryl 430 on yeast has been studied. They have reported that the first effect is the production of modulations and that this is followed by the production of mutations, a two-step process of mutation which parallels the two-step process of carcinogenicity.

BIRD and FAHMY (1953), using the Muller-5 technique for detecting lethal mutations in Drosophila, studied the carcinogenicity, tumor-inhibiting power and mutagenicity of eleven compounds. They found these three characteristics to be essentially parallel.

In this article, additional evidence for the mutagenicity for *E. coli* of two carcinogens, methylcholanthrene and 1,2,5,6-dibenzanthracene-9,10-α,β-endosuccinate (DBAS), and evidence for the mutagenicity of three others, 3,4-benzpyrene, 9,10-bishydroxymethyl-1,2-benzanthracene (BB) and *o*-aminoazotoluene is presented.

EXPERIMENTAL

The methods used in this work parallel those of WITKIN (1947), which were in turn based on those of DEMEREC (1946) and DEMEREC and LATARJET (1946). Suspensions or cultures of *E. coli* B/r were assayed for mutants that were resistant to T1 bacteriophage both before and after being subjected to the chemical agents. Both the *E coli* culture and the bacteriophage strain were obtained from DR. WITKIN.

Concentrated cell suspensions were prepared by collecting cells by centrifugation from four 25-ml cultures in M9 medium (for composition see WITKIN 1947) that had been incubated for 48 hours at 37°C with shaking. The cells were washed once and resuspended in 10 ml of buffered distilled water (Standard Methods For the Examination of Dairy Products, 1948).

In the tests, 2 ml of the concentrated cell suspension were added to 2 ml of the stock carcinogen suspension and 16 ml of suspending medium. This mixture, together with a control consisting of 2 ml of the cell suspension and 18 ml of suspending medium, was incubated at 37°C with shaking. Total cell counts and counts of B/r/1 mutants were made before incubation and after various intervals of incubation. Mutagenicity was judged by the ratio of mutants in the experimental and control cultures after incubation. Control experiments were run with a noncarcinogen, anthracene.

Except for the water-soluble carcinogen, DBAS, the stock carcinogen suspensions contained 2.04 mg/ml of distilled water. These suspensions were stored in the refrigerator in the dark. Because of low solubility of these carcinogens the bacteria were exposed to saturated solutions containing extra carcinogen in suspension. The stock solution of the water-soluble carcinogen contained 1.0 mg/ml. Total cell counts were made by plating on nutrient agar.

For determining the concentration of B/r/1 cells, 0.1 ml portions of the suspension or culture were deposited on the surfaces of ten nutrient agar plates that had been prepared 24 hours previously and stored at room temperature with porous clay tops. To each plate was added 0.1 ml of a freshly prepared

bacteriophage suspension with a titer of 5×10^9 to 1×10^{10}. This was thoroughly mixed with the culture with a glass rod and the mixture was spread over the surface of the agar. The plates were allowed to dry for 15 to 20 minutes, and were then inverted and incubated at 37°C for 48 hours.

Experiments were considered valid only if the number of mutants in the control, "background number," was small. The mutagenic effect was measured by dividing the percent B/r/1 cells in the experimental culture by the percent B/r/1 cells in the control. It was assumed that if no mutagenic action occurred the ratio would be 1.00. All ratios that have been considered to indicate mutagenicity have been distinctly greater than this figure.

Experiments with 9,10-bishydroxymethyl-1,2-benzanthracene (BB)

The first compound to be tested was BB. It was kindly supplied by Dr. Alexander Haddow of the Royal Cancer Hospital, London. It had been synthesized by Kennaway and had been shown to display marked carcinogenic activity for mice (Badger and Cook 1940). This compound was selected because it has greater solubility than other compounds with the benzanthracene nucleus. The results of a series of experiments with this compound are shown in table 1.

In the first test the suspending solution used was buffered distilled water. After an incubation period of three hours the total count on the control suspension had dropped to 28.8 percent of that before incubation and the total count on the suspension with the carcinogen had dropped to 9.7 percent of the original, indicating toxicity of the carcinogen. The ratio of 6.95 obtained indicates definite mutagenicity, apparently in the form of "zero point" mutations since growth can hardly be expected to have taken place within a three-hour period in buffered distilled water. In the second test M9 medium was used as a suspending medium. After 2½ hours the total count had fallen to 8.2 percent of the original although the count on the control was 92 percent of the original, and the mutant ratio was 12.9. There was not only an increase in the percentage of mutants in the suspensions but also a significant increase in the actual number of mutants ($P < .02$). This is considered to be important since, as critics of experiments on mutagenesis have so frequently pointed out, an increase in percentage of mutants might so easily be due to selective toxicity.

In the next test the same experimental setup was used, with total counts and counts of B/r/1 mutants being made after 2, 4, 12 and 24 hours of incubation. From the table it can be seen that toxic effects of the carcinogen were evident for the first 4 hours, with the total count dropping to 2.2 percent of the original after this period of time. Growth evidently began at about this time since the count after 12 hours was considerably increased. The maximum mutant ratio occurred at the same time at which the maximum toxicity was evident and decreased after growth began. This result was unexpected since it was anticipated that growth would give an opportunity for "end point" mutations to become evident. In an experiment performed in an attempt to partially explain these results it was found that cells that had been

TABLE 1

Mutagenicity as measured by production of E. coli B/r/1 mutants.

	Period of exposure	Total count per ml	% of original count	B/r/1 mutants per ml	B/r/1 mutants per 10^6 cells	Experimental/ control ratio
A. Tests with BB* in buffered distilled water						
Control	0	5.95×10^7		21	35.30	
Control	3 hr.	1.71×10^7	28.8	6	35.10	
Experimental	3 hr.	5.75×10^6	9.7	14	244.00	6.95
B. Tests with BB in M9 medium						
Control	0	5.7×10^8		650	114.00	
Control	2½ hr.	5.3×10^8	92	635	120.00	
Experimental	2½ hr.	4.7×10^7	8.2	727	550.00	12.90
C. Tests with BB in M9 medium						
Control	0	4.9×10^9		187	3.82	
Control	24 hr.	7.4×10^{10}	1510	2970	4.01	
Experimental	2 hr.	4.6×10^8	9.4	130	28.20	7.39
Experimental	4 hr.	1.1×10^8	2.2	52	47.30	12.38
Experimental	12 hr.	1.6×10^9	32.6	245	15.40	3.84
Experimental	24 hr.	3.3×10^9	67.4	415	12.60	3.14
D. Tests with methylcholanthrene in M9 medium						
Control	0	5.5×10^8		1802	328.00	
Control	3 hr.	4.6×10^8	83.6	1625	353.00	
Experimental	3 hr.	4.3×10^8	78.2	1607	373.00	1.06
E. Tests with methylcholanthrene in M9 medium + 20% sheep plasma						
Control	0	3.2×10^8		429	134.00	
Control	4 hr.	12.5×10^6	3.9	18	144.00	
Experimental	4 hr.	3.7×10^6	1.1	20	541.00	3.76
F. Tests with anthracene (noncarcinogenic control) in M9 medium						
Control	0	6.7×10^8		702	105.00	
Control	3 hr.	14.1×10^8	210	1418	101.00	
Experimental	3 hr.	12.4×10^8	185	1322	107.00	1.06

*9,10-bishydroxymethyl-1,2-benzanthracene.

incubated for 36 hours in the presence of the carcinogen were resistant to its toxic effects. If it be assumed that surviving cells are likewise resistant to the mutagenic effects of the carcinogen then it may be postulated that the rate of production of mutants decreases as soon as growth begins to occur in the culture. If this decrease in rate of production of mutants is accompanied by the development of selective conditions which favor the wild type over the mutants then results similar to those obtained might be expected.

A series of control experiments was next run to eliminate as far as possible the possibility that the increased mutant ratios were a result of something other than mutagenesis. Comparative toxicity tests with one of the spontaneous B/r/1 mutants and with the parent B/r strain indicated that the mutant was

not more resistant to the toxic effects of the carcinogen. Before the toxicity tests were made the mutant culture was subjected to extensive tests to prove that it was a pure B/r/1 strain. A suspension of the mutant in buffered distilled water that was incubated with shaking for three hours in the presence of the carcinogen yielded 23.8 percent survivors as compared to 46.2 percent in the control without carcinogen. In another test in which a suspension containing equal numbers of B/r and B/r/1 cells was incubated for three hours in the presence and in the absence of the carcinogen, 48.0 percent B/r/1 cells were found in the suspension with carcinogen and 46.5 percent in the control without carcinogen. The possibility always remains after experiments of this type that the comparative toxicities might be different in a suspension in which a very small percentage of B/r/1 cells was present, or that other B/r/1 strains might be more resistant to the carcinogen.

Growth curves of the B/r and B/r/1 cultures in M9 medium were apparently identical. This indicates that the results obtained were not due to differences in growth rates, although it is possible that the growth rates might be different in a mixed culture or in the presence of the carcinogen.

Lack of toxicity of the carcinogen for the bacteriophage was indicated by the fact that when equal amounts of carcinogen suspension, 24-hr. broth culture of *E. coli* B/r, and bacteriophage suspension were mixed and spread on the surfaces of agar plates no reduction in the number of plaques was found as compared with control plates made by substituting buffered distilled water for the carcinogen suspension.

As can be seen in table 1 the non-carcinogenic control, anthracene, yielded a mutant ratio of 1.06 after an exposure period of three hours in M9 medium and cannot, therefore, be judged to be mutagenic.

Experiments with 20-methylcholanthrene

A mutant ratio of 1.06 was obtained for methylcholanthrene (Eastman Kodak Co.) after an exposure period of three hours in M9 medium (table 1). In this test the methylcholanthrene showed little or no toxicity. Total counts showed that 78.2 percent of the cells survived the exposure period as compared to 83.6 percent of the cells in the control without methylcholanthrene. At this point it was postulated that the lack of activity of the methylcholanthrene might be due to its insolubility. Therefore the experiment was repeated with enough of the M9 medium being replaced by sheep plasma to give 20 percent plasma in the final suspension. It was found that after 4 hours incubation the methylcholanthrene was definitely toxic and a mutant ratio of 3.76 was obtained. It is assumed, although not proven, that the effect of the plasma was to increase the solubility of the methylcholanthrene. The lack of solubility of methylcholanthrene may explain LATARJET's (1948) failure to obtain an increase in number of bacteriophage-resistant mutants of *E. coli* B. BARRATT and TATUM (1951) used Tween 80 in the medium as a solvent and were able to demonstrate mutagenicity in Neurospora.

TABLE 2

Mutagenicity as measured by production of E. coli B/r/1 mutants.

	Exposure medium	Period of exposure	Total count per ml	B/r/1 mutants per ml	B/r/1 per 10^6 cells	Experimental/ control ratio
		A. Tests with 3:4-benzpyrene and DBAS*				
Control	Buffered distilled water	4 hr.	4.5×10^8	80	17.80	
Control	Buffered distilled water	16 hr.	5×10^6	16	320.00	
Control	Nutrient broth	4 hr.	4.3×10^{10}	662	1.54	
Benzpyrene	Buffered distilled water	4 hr.	4.5×10^8	312	69.30	3.90
Benzpyrene	Buffered distilled water	16 hr.	3×10^6	29	967.00	3.02
Benzpyrene	Nutrient broth	4 hr.	19.5×10^9	1485	7.60	4.95
DBAS*	Buffered distilled water	4 hr.	4.3×10^8	404	94.00	5.30
DBAS	Buffered distilled water	16 hr.	3.3×10^6	43	1300.00	4.06
DBAS	Nutrient broth	4 hr.	14.3×10^9	2101	14.90	9.66
		B. Tests with o-aminoazotoluene and anthracene (noncarcinogenic control)				
Control	Buffered distilled water	6 hr.	4×10^8	17	4.25	
Control	Buffered distilled water	16 hr.	9×10^7	14	15.60	
Control	Nutrient broth	4 hr.	1.1×10^8	39	35.50	
o-aminoazotoluene	Buffered distilled water	6 hr.	6×10^8	45	7.50	1.76
o-aminoazotoluene	Buffered distilled water	16 hr.	9.8×10^7	26	26.50	1.63
o-aminoazotoluene	Nutrient broth	4 hr.	9×10^7	61	67.80	1.91
Anthracene	Buffered distilled water	6 hr.	5×10^8	17	3.40	0.80
Anthracene	Buffered distilled water	16 hr.	1×10^8	13	13.00	0.83
Anthracene	Nutrient broth	4 hr.	1.2×10^8	35	29.20	0.83

*1,2,5,6-dibenzanthracene-α,β-endosuccinate.

Experiments with 3,4-benzpyrene, 1,2,5,6-dibenzanthracene-α,β-endosuccinate (DBAS) and o-aminoazotoluene

The 3,4-benzpyrene was obtained from A. D. MACKEY, Pure Chemicals-Metals and Minerals, 198 Broadway, N. Y., and the *o*-aminoazotoluene (2-amino-5-azotoluene) from the Eastman Kodak Co. The 1,2,5,6-dibenzanthracene-α,β-endosuccinate was synthesized according to the method of Bachmann and Kloetzel (1938).

Mutant ratios were determined for 3,4-benzpyrene and DBAS after four and sixteen hours of incubation in buffered distilled water and for *o*-aminazotoluene after six and sixteen hours of incubation. Mutant ratios were also determined for each substance after 4 hours incubation in nutrient broth.

In these studies counts of B/r/1 mutants in the controls were made only after the same periods of incubation as in the controls since counts on the suspensions previous to incubation did not appear to give essential information. Although in the previous studies the " background number " of mutants had remained constant in spite of incubation, in these studies the " background number " increased between the 4th or 6th and 16th hours of incubation. This does not appear to have obscured the effects of the test substances.

From the results in table 2 it can be seen that the water-soluble carcinogen, DBAS, was the strongest mutagen, especially when it is recalled that it was used in a considerably lower concentration. The 3,4-benzpyrene was also a strong mutagen but the *o*-aminoazotoluene was the weakest mutagen of the carcinogens tested. The anthracene (noncarcinogenic) again proved to be nonmutagenic.

Total counts showed no toxicity for the benzpyrene and the DBAS in distilled water after the 4-hour exposure but slight toxicity after 16 hours. Counts on the suspension with *o*-aminoazotoluene in distilled water were higher than counts on the control. Inhibition of growth was evident in all the 4-hr. nutrient broth cultures containing carcinogens but not in that containing anthracene. The degree of inhibition was parallel to the degree of mutagenicity, being strongest for the DBAS and weakest for the *o*-aminoazotoluene.

As can be seen in table 2, the mutant ratios for the suspensions in buffered distilled water were slightly lower after 16 hours than after 4 or 6 hours, in every case. The mutant ratios for the nutrient broth cultures were distinctly higher than those for the water suspensions, possibly indicating " end point " mutations in the growing cultures.

Growth curves were determined for the parent B/r culture and for a B/r/1 strain (a different strain from that used in the studies with BB) in nutrient broth. The rate of growth was identical for the first six hours, after which the parent B/r culture grew more rapidly and reached a higher maximum population. The effects of each of the carcinogens on the growth of the parent B/r culture and the B/r/1 strain were next determined. Each of the carcinogens was inhibitive to the B/r/1 strain but not to the parent B/r culture. These results appear to indicate that both growth rates and the selective effects of

the carcinogens favored the parent type over the mutants in the nutrient broth cultures. This enhances the evidence for mutagenicity.

Benzpyrene was shown to have no effect on the plaque count produced by a bacteriophage suspension or on its ability to select B/r/1 mutants when the suspension was subjected to the carcinogen for 4 hours.

Fifteen of the B/r/1 mutants from the various experiments were tested for purity and then tested for resistance to T1, T2, and T5 bacteriophages. All were resistant to T1 and sensitive to T2. Six were resistant to T5. The abilities of all 15 mutants to grow in a synthetic medium without added tryptophane were tested. The results with fourteen agreed with the finding of DEMEREC and FANO (1945) that only the strains that are resistant to both T1 and T5 bacteriophages can grow without added tryptophane. One strain that was resistant to both bacteriophages failed, upon repeated tests, to grow in the absence of tryptophane, although it grew well in its presence.

SUMMARY

Evidence has been presented that the carcinogens, 20-methylcholanthrene, 1,2,5,6-dibenzanthracene-α,β-endosuccinate (DBAS), 3,4-benzpyrene, 9,10-bishydroxymethyl-1,2-benzanthracene (BB) and *o*-aminoazotoluene increased the rate of production of B/r/1 mutants from *Escherichia coli* B/r. A noncarcinogen, anthracene, had no effect. Mutagenesis was apparently demonstrated in buffered distilled water suspensions, in M9 medium, and in nutrient broth cultures, the strongest effects being in the latter. 3,4-Benzpyrene, DBAS, and BB appeared to be strong mutagens. Mutagenic effects of methylcholanthrene could only be demonstrated when 20 percent sheep plasma was added to the suspending M9 medium, the effect probably being one of increasing the solubility of the carcinogen. *o*-Aminoazotoluene was weakly mutagenic. The mutagenicity of the compounds tested appeared to parallel their reported carcinogenicity when their solubility was taken into account. Toxicity and growth rate studies with the parent B/r culture and with two of the B/r/1 mutants supported the view that the effects observed represent true mutagenesis.

LITERATURE CITED

American Public Health Association, 1948 Standard Methods for the Examination of Dairy Products, Ninth Edition. New York, N. Y.

AUERBACH, C., and J. M. ROBSON, 1944 Production of mutations by allyl isothiocyanate. Nature **154:** 81–82.

BACKMANN, W. E., and M. C. KLOETZEL, 1938 The reaction between maleic anhydride and polycyclic hydrocarbons. J. Amer. Chem. Soc. **60:** 481–485.

BADGER, G. M., and J. W. COOK, 1940 The Synthesis of growth inhibitory polycyclic compounds. Part II. J. Chem. Soc. (Lond.) 409–412.

BARRATT, R. W., and E. L. TATUM, 1951 An evaluation of some carcinogens as mutagens. Cancer Res. **11:** 234.

BIRD, M. J., and O. G. FAHMY, 1953 Cytogenetic analysis of the action of carcinogens and tumour inhibitors in *Drosophila melanogaster*. I. 1,2,3,4-diepoxybutane. Proc. Roy. Soc., Lond. B **140:** 556–578.

BONNER, D., 1946 Biochemical mutants in Neurospora. Cold Spring Harbor Symp. Quant. Biol. **11:** 14–24.

BOYLAND, E., and E. S. HORNING, 1949 The induction of tumours with nitrogen mustards. Brit. J. Cancer **3:** 118–123.

BRYSON, V., 1948 The effects of nitrogen mustard on *Escherichia coli*. J. Bact. **56:** 423–433.

BURDETTE, W. J. 1952 Effect of nitrogen mustard on tumor incidence and lethal mutation rate in *Drosophila*. Cancer Res. **12:** 366–368.

DEMEREC, M., 1946 Induced mutations and possible mechanisms of the transmission of heredity in *Escherichia coli*. Proc. Nat. Acad. Sci. **32:** 36–46.

1947a Mutations in Drosophila induced by a carcinogen. Nature **159:** 604.

1947b Production of mutations in Drosophila by treatment with some carcinogens. Science **105:** 634.

DEMEREC, M., and V. FANO, 1945 Bacteriophage-resistant mutants in *Escherichia coli*. Genetics **30:** 119–136.

DEMEREC, M., and R. LATARJET, 1946 Mutations in bacteria induced by radiation. Cold Spring Harbor Symp. Quant. Biol. **11:** 38–50.

GRIFFIN, A. C., E. L. BRANDT and E. L. TATUM, 1951 Induction of tumors with nitrogen mustard. Cancer Res. **11:** 253.

HESTON, W. E., 1949 Induction of pulmonary tumors in strain A mice with methylbis (β-chloroethyl) amine hydrochloride. J. Nat. Cancer Inst. **10:** 125–130.

1950 Carcinogenic action of the mustards. J. Nat. Cancer Inst. **11:** 415–423.

HOROWITZ, N. H., M. B. HOULAHAN, M. G. HUNGATE and B. WRIGHT, 1946 Mustard gas mutations in *Neurospora*. Science **104:** 233–234.

LATARJET, R., 1948 Production d'une mutation bacteriènne par des substances cancérigènes ou non. C. R. Soc. Biol. **142:** 453–455.

LATARJET, R., C. ELIAS and N. P. BUU-HOI, 1949 Production d'une mutation bacteriènne par des cancérigènes hydrosolubles. C. R. Soc. Biol. **143:** 776–778.

MAISIN, J., 1951 Mutations, cancer expérimental et environnement. Scientia **86:** 20–31.

MAISIN, J., G. LAMBERT and E. VAN DUYSE, 1951 Mutations et modulations chez *Saccharomyces cerevisiae*. Leurs rapports avec la cancerisation expérimentale. Rév. Belge Path. Med. Exp. **21:** 52–66.

MITRA, K. K., and M. K. SUBRAMANIAM, 1950 Studies on the mutagenic action of chemical and physical agencies on yeasts. III. Further observations on genetical effects of chrysene on yeasts. J. Indian Inst. Sci. **32A:** 113–128.

SCHERR, G. H., and R. H. WEAVER, 1949 The induction of bacteriophage-resistant mutations of *Escherichia coli* by a carcinogenic agent. Abstracts of Papers, Society of American Bacteriologists, 16.

TATUM, E. L., 1946 Induced biochemical mutations in bacteria. Cold Spring Harbor Symp. Quant. Biol. **11:** 278–284.

1947 Chemically induced mutations and their bearing on carcinogenesis. Ann. N. Y. Acad. Sci. **49:** 87–97.

WITKIN, E. M., 1947 Mutations in *Escherichia coli* induced by chemical agents. Cold Spring Harbor Symp. Quant. Biol. **12:** 256–269.

PANMIXIA WITH TETRASOMIC AND HEXASOMIC INHERITANCE *

J. H. BENNETT

Department of Genetics, Cambridge University, Cambridge, England

Received June 20, 1953

BECAUSE of the greater number of genotypes and of modes of gamete formation, any genetic analysis is much more complex when the nature of inheritance is polysomic rather than disomic. Consequently, whilst random mating in populations of disomic organisms has been considered in some detail (for a list of references see BENNETT 1954), there have been few parallel discussions for polysomic forms. HALDANE (1930) first considered random mating with a single polysomic locus under the assumption of random chromosome segregation. The nature of polysomic inheritance at a single locus has since been clarified (FISHER and MATHER 1943) and recently, GEIRINGER (1949) has given a complex treatment of random mating at a single polysomic locus taking account of double reduction. I have also given an independent approach for the case of a tetrasomic locus (BENNETT 1949). In the present article, I consider random mating with linkage in tetrasomic and hexasomic inheritance. The assumptions made are that the generations do not overlap, that the same conditions exist for the two sexes and that there is neither mutation nor selection.

UNIFACTORIAL DISTRIBUTIONS

For a single factor, the effect of a generation of random mating can be expressed as a linear transformation of the gametic frequencies. The results for tetrasomic inheritance may be summarized as follows. Suppose that there are any number of alleles $A, a, a', \ldots$ at the given locus and that these are present in the original population with frequencies $p(A)$, $p(a)$, $p(a')$, etc. These frequencies are constant in the absence of selection and mutation. If p_n denotes the frequency of occurrence of a given gametic genotype in the pooled gametic output of the nth generation of random mating and α is the amount of double reduction at the given locus, typical principal components of frequency (FISHER 1949, p. 30) are as follows.

$$L_n = (2+\alpha)\, p_n(A^2) - 2(1-\alpha)\, p(A)^2 - 3\alpha p(A) \qquad (1)$$

$$M_n = (2+\alpha)\, p_n(Aa) - 4(1-\alpha)\, p(A)\, p(a) \qquad (2)$$

Each of these principal components corresponds with the latent root $(1-\alpha)/3$, i.e.,

$$L_n = [(1-\alpha)/3]^n L_0 \text{ and } M_n = [(1-\alpha)/3]^n M_0$$

* Part of the cost of the accompanying mathematical formulae has been paid by the GALTON and MENDEL MEMORIAL FUND.

These results are equivalent to Geiringer's equations (21) but there is a slight difference in expression. In the present article no distinction is made between genotypes Aa and aA, and $p(Aa)$ is used to denote the total frequency of both these genotypes.

With a single locus and disomic inheritance, the distribution of zygotic genotypes reaches the equilibrium state after one generation of random mating (HARDY 1908). This is not the case with tetrasomic inheritance. Not only is the rate of approach to equilibrium dependent upon the amount of double reduction but so are the equilibrium distributions themselves. It is only in the case of no double reduction that the distribution of gametes approaches the state of independence in which $p(A^2) = p(A)^2$, $p(Aa) = 2p(A)p(a)$, etc. To compare the rates of approach to equilibrium at different loci we may compare the values of $\log_e[3/(1-\alpha)]$.

The equilibrium distribution of zygotic genotypes is given by the expansion of

$$\left\{3\alpha \sum_a p(a)a^2 + 2(1-\alpha)\left[\sum_a p(a)a\right]^2\right\}^2 / (2+\alpha)^2 \qquad (3)$$

where the summations are taken over all alleles at the given locus (cf. GEIRINGER's equations (23)). If $\alpha = 0$, this is

$$\left[\sum_a p(a)a\right]^4$$

(cf. HALDANE 1930, p. 363).

Now consider the result of random mating for a single hexasomic locus. If β denotes the amount of double reduction at the given locus, typical principal components of frequency are as follows (cf. GEIRINGER's equations (28)).

$$L_n = (9+\beta)(9+2\beta)p_n(A^3) - 27(1-\beta)(3-\beta)p(A)^3 - 45\beta(3-\beta)p(A)^2 - 20\beta^2 p(A) \qquad (4)$$

$$M_n = (9+\beta)(9+2\beta)p_n(A^2a) - 81(1-\beta)(3-\beta)p(A)^2p(a) - 45\beta(3-\beta)p(A)p(a) \qquad (5)$$

$$N_n = (9+\beta)(9+2\beta)p_n(Aaa') - 162(1-\beta)(3-\beta)p(A)p(a)p(a') \qquad (6)$$

Each of these principal components corresponds with the latent root $(6-2\beta)/15$ and so to compare the rates of approach to equilibrium at different loci, we may compare the values of $\log_e[15/(6-2\beta)]$. At equilibrium, the genotypic distribution of zygotes is given by the expansion of

$$\left\{27(1-\beta)(3-\beta)\left[\sum_a p(a)a\right]^3 + 45\beta(1-\beta)\left[\sum_a p(a)a^2\right]\sum_a p(a)a + 20\beta^2 \sum_a p(a)a^3\right\}^2 / \left[(9+\beta)(9+2\beta)\right]^2 \qquad (7)$$

each summation being taken over all alleles at the locus.

LINKAGE WITH TETRASOMIC INHERITANCE

With disomic inheritance, the genotypes of all individuals which are heterozygous at a given number of linked loci can be transformed into one another by gene substitutions. Whilst this is not the case with polysomic inheritance, the heterogenic genotypes may be classified into a number of isomorphic sets such that each genotype of a set can be transformed into any other of the same set by a number of gene substitutions. All genotypes of any isomorphic set have equivalent gametic frequencies. This is in accordance with the generalized form of the Mendelian law of heredity, " The gametic frequencies are invariant in respect of any gene substitution applied systematically to the genic content of an organism and of the gametes it produces " (FISHER 1947, p. 58).

TABLE 1

Modes of gamete formation for two loci in a tetrasomic organism. Parental genotype $a_1b_1/a_2b_2/a_3b_3/a_4b_4$.

Mode of formation	Typical gamete
1	a_1b_1/a_2b_2
2	a_1b_1/a_1b_1
3	a_1b_1/a_2b_3
4	a_1b_1/a_1b_2
5	a_1b_1/a_2b_1
6	a_1b_2/a_3b_4
7	a_2b_1/a_3b_1
8	a_1b_2/a_1b_3
9	a_1b_2/a_2b_3
10	a_1b_2/a_1b_2
11	a_1b_2/a_2b_1

Taking together the isomorphic sets of genotypes which are related by permutation of loci, we have a classification according to genera (loc. cit., p. 70).

With two linked loci and disomic inheritance, there are only two modes of gamete formation and these correspond to recombinant and non-recombinant gametes. The gametic output of an individual heterozygous at each of two linked loci can therefore be specified in terms of a single parameter, the recombination fraction. With tetrasomic inheritance at two linked loci, eleven modes of gamete formation can be distinguished. We shall adopt FISHER's (1947) classification of these modes of formation (table 1). The respective frequencies of occurrence of these modes of gamete formation will be denoted by f_i ($i = 1, 2, \ldots, 11$) where

$$\sum_{i=1}^{11} f_i = 1.$$

The gametic output of any tetrasomic individual heterogenic at two linked loci can therefore be specified linearly in terms of these eleven parameters.

We shall now consider the chromosome or gene-combination frequencies upon random mating. Suppose that two alleles are available at both of the linked loci and let these be denoted by A,a and B,b. For those genotypes with

only one allele at either locus, the frequency of occurrence of any pair of genes at these two loci on one chromosome is, of course, the same in the gametic output as it is in the parental genotype itself. With one exception, this is not true of genotypes with two alleles at both loci. The exceptional genotype is biduplex neutral, *AB/Ab/aB/ab*. For all other genotypes with two alleles at both loci, the frequency of occurrence of any pair of genes on one chromosome in the gametes is some linear function of the recombination fraction. These frequencies relevant to the gametic output from one representative genotype of each of the five genera are shown in table 2. The recombination fraction is denoted by y.

Any gene-combination has the same frequency of occurrence in the zygotes of a given generation as in the gametic output of the preceding generation. The relations between the frequencies of gene-combinations in the gametic output of a given generation and in the zygotes of the preceding generation are contained in table 2. If w_n denotes the frequency of a given zygotic genotype in the nth generation of random mating and p_n that of a given gametic genotype,

TABLE 2

Frequencies of gene-combinations in gametic output of tetraploids.

Typical parental genotype	Number of genotypes in genus	Frequencies of gene-combinations in the gametic output				÷
		AB	*Ab*	*ab*	*aB*	
AB/ab/ab/ab	4	$1-y$	y	$3-y$	y	4
Ab/aB/ab/ab	4	y	$3-y$	$6+y$	$3-y$	12
AB/Ab/ab/ab	4 + 4	$3-2y$	$3+2y$	$6-2y$	$2y$	12
AB/AB/ab/ab	2	$3-2y$	$2y$	$3-2y$	$2y$	6
AB/Ab/aB/ab	1	1	1	1	1	4

or of a given gene-combination (chromosome), amongst the total gametic output of this same generation, then from table 2 we can immediately obtain the following relation.

$$
\begin{aligned}
p_{n+1}(AB) - p_n(AB) = {} & \\
& (y/12)[-3w_{n+1}(AB/ab/ab/ab) + 3w_{n+1}(Ab/Ab/Ab/aB) \\
& \quad - 3w_{n+1}(AB/AB/AB/ab) + 3w_{n+1}(Ab/aB/aB/aB) \\
& \quad - w_{n+1}(AB/Ab/Ab/ab) + w_{n+1}(Ab/aB/ab/ab) \\
& \quad - w_{n+1}(AB/aB/aB/ab) + w_{n+1}(AB/AB/Ab/aB) \\
& \quad - 2w_{n+1}(AB/Ab/ab/ab) + 2w_{n+1}(Ab/Ab/aB/ab) \\
& \quad - 2w_{n+1}(AB/AB/aB/ab) + 2w_{n+1}(AB/Ab/aB/aB) \\
& \quad - 2w_{n+1}(AB/aB/ab/ab) + 2w_{n+1}(AB/Ab/Ab/aB) \\
& \quad - 2w_{n+1}(AB/AB/Ab/ab) + 2w_{n+1}(Ab/aB/aB/ab) \\
& \quad - 4w_{n+1}(AB/AB/ab/ab) + 4w_{n+1}(Ab/Ab/aB/aB)]. \qquad (8)
\end{aligned}
$$

As the zygotes of the $(n+1)$th generation are formed by the random union of gametes produced from the nth generation, the zygotic frequencies are expressible as quadratic functions of the gametic frequencies of the previous generation. For example,

$$w_{n+1}(AB/ab/ab/ab) = 2p_n(AB/ab)p_n(ab/ab)$$
$$w_{n+1}(AB/Ab/ab/ab) = 2p_n(AB/Ab)p_n(ab/ab) + 2p_n(AB/ab)p_n(Ab/ab) \quad (9)$$
$$w_{n+1}(AB/AB/ab/ab) = 2p_n(AB/AB)p_n(ab/ab) + p_n(AB/ab)p_n(AB/ab).$$

Making these substitutions in (8), we arrive, after some simplification, at the following equation.

$$p_{n+1}(AB) = p_n(AB) + (y/6)[4p(A)p(B) - 4p_n(AB) + p_n(Ab/aB) - p_n(AB/ab)] \quad (10)$$

If $y = 0$, then $p_n(AB) = p_0(AB)$ as for a single locus. Now gametes of genotypes *AB/ab* and *Ab/aB* can be formed only from individuals that are digenic at both loci. From the gametic matrices for such individuals (FISHER 1947) we find that

$$\begin{aligned} p_n(Ab/aB) - p_n(AB/ab) = {} & \\ (1/12)[2(f_1 - f_{11}) + f_3 - f_9][& -3w_n(AB/ab/ab/ab) + 3w_n(Ab/Ab/Ab/aB) \\ & - 3w_n(AB/AB/AB/ab) + 3w_n(Ab/aB/aB/aB) \\ & - w_n(AB/Ab/Ab/ab) + w_n(Ab/aB/ab/ab) \\ & - w_n(AB/aB/aB/ab) + w_n(AB/AB/Ab/aB) \\ & - 2w_n(AB/Ab/ab/ab) + 2w_n(Ab/Ab/aB/ab) \\ & - 2w_n(AB/AB/aB/ab) + 2w_n(AB/Ab/aB/aB) \\ & - 2w_n(AB/aB/ab/ab) + 2w_n(AB/Ab/Ab/aB) \\ & - 2w_n(AB/AB/Ab/ab) + 2w_n(Ab/aB/aB/ab) \\ & - 4w_n(AB/AB/ab/ab) + 4w_n(Ab/Ab/aB/aB)] \end{aligned} \quad (11)$$

Hence, by reason of (8),

$$p_n(Ab/aB) - p_n(AB/ab) = [2(f_1 - f_{11}) + f_3 - f_9] \times [p_n(AB) - p_{n-1}(AB)]/y \quad (12)$$

Hence from (10) we obtain the following relation.

$$p_{n+1}(AB) = p_n(AB) + (1/6)[2(f_1 - f_{11}) + f_3 - f_9][p_n(AB) - p_{n-1}(AB)] + (2y/3)[p(A)p(B) - p_n(AB)] \quad (13)$$

Putting $r_n(AB) = p(A)p(B) - p_n(AB)$

then $r_{n+1}(AB) - (1 + \delta)r_n(AB) + (\gamma + \delta)r_{n-1}(AB) = 0$

where $\gamma = 2y/3$

and $\delta = -\gamma + [2(f_1 - f_{11}) + f_3 - f_9]/6.$

The solution of this recurrence equation is

$$r_n(AB) = -(C_n + D_n)r_0(AB) + D_n[p_0(Ab/aB) - p_0(AB/ab)]$$

where C_n and D_n are given by

$$\begin{bmatrix} C_n \\ D_n \end{bmatrix} = \begin{bmatrix} 1 & 1 \\ \delta & -\gamma \end{bmatrix}^n \begin{bmatrix} -1 \\ 0 \end{bmatrix}$$

The matrix $\begin{bmatrix} 1 & 1 \\ \delta & -\gamma \end{bmatrix}$ has positive latent roots,

$$\lambda = (\tfrac{1}{2})\{1 + \delta + [(1+\delta)^2 - 4(\gamma+\delta)]^{1/2}\}$$
$$\mu = (\tfrac{1}{2})\{1 + \delta - [(1+\delta)^2 - 4(\gamma+\delta)]^{1/2}\}$$

These latent roots correspond with principal components

$$L_n = 2\gamma C \div \{1 - \delta - [(1+\delta)^2 - 4(\gamma+\delta)]^{1/2}\} D_n \quad (14)$$
$$M_n = 2\gamma C \div \{1 - \delta + [(1+\delta)^2 - 4(\gamma+\delta)]^{1/2}\} D_n \quad (15)$$

such that

$$L_n = -2\gamma\lambda^n$$
$$M_n = -2\gamma\mu^n$$

If $4(\gamma+\delta)$ is less than $(1+\delta)^2$, there will be a real value for θ such that $4(\gamma+\delta)\cosh^2\theta = (1+\delta)^2$ and we may then write

$$\lambda^n = (\gamma+\delta)^{n/2}\exp(n\theta)$$
$$\mu^n = (\gamma+\delta)^{n/2}\exp(-n\theta).$$

It follows that

$$C_n = -\frac{1}{2}(1-\delta)(\gamma+\delta)^{(n-1)/2}\frac{\sinh n\theta}{\sinh\theta} - (\gamma+\delta)^{n/2}\cosh n\theta$$

$$D_n = \gamma(\gamma+\delta)^{(n-1)/2}\frac{\sinh n\theta}{\sinh\theta}$$

As $(\gamma+\delta)^{n/2}\exp\theta$ is less than unity for all possible values of γ other than $\gamma = 0$, it follows that

$$\lim_{n\to\infty} C_n = 0$$

$$\lim_{n\to\infty} D_n = 0.$$

$$\text{Hence } \lim_{n\to\infty} p_n(AB) = p(A)\,p(B). \quad (16)$$

A comparison of the rates of approach to equilibrium at different pairs of linked loci may be made by comparing the values of

$$-\log_e\lambda = \log_e\{(\tfrac{1}{2})(1+\delta) + [(\tfrac{1}{4})(1-\delta)^2 - \gamma]^{1/2}\}.$$

For two closely linked loci in chromosomes which form only bivalents

$$f_1 = 1 - 2y,\ f_3 = 2y,$$
$$f_2 = f_4 = f_5 = f_6 = f_7 = f_8 = f_9 = f_{10} = f_{11} = 0.$$

In this case, we have approximately, when n is large,

$$C_n = -\exp(-ny)$$
$$D_n = y\exp(-ny)$$

so that

$$p_n(AB) = p(AB) + g(0)[1 - \exp(-ny)] + y\,h(0)\exp(-ny).$$

It follows that the equilibrium frequencies are the products of the equilibrium frequencies for the separate loci, i.e., at equilibrium, the factors are associated at random. For example,

$$\lim_{n\to\infty} p_n(AB/AB) = [9/(2+\alpha)(2+\alpha')]\,[\alpha p(A) + 2(1-\alpha)p(A)^2/3] \times [\alpha' p(B) + 2(1-\alpha')p(B)^2/3]$$

$$\lim_{n\to\infty} p_n(AB/Ab) = [9/(2+\alpha)(2+\alpha')]\,[\alpha p(A) + 2(1-\alpha)p(A)^2/3] \times [4(1-\alpha')p(B)p(b)/3]$$

and so on, where $\alpha = f_2 + f_4 + f_8 + f_{10}$ and $\alpha' = f_2 + f_5 + f_7 + f_{10}$, are the frequencies of double reduction at the first and second loci respectively.

LINKAGE WITH HEXASOMIC INHERITANCE

An analysis similar to that of the preceding section may be carried out for hexasomic inheritance. There are sixty hexasomic genotypes digenic at both of two linked loci and these may be classified into twelve genera (FISHER 1947, p. 69). The inheritance is characterized by forty modes of gamete formation. The enumeration which will be adopted for these is the same as I have used elsewhere (BENNETT 1953).

TABLE 3

Frequencies of gene-combinations in gametic output of hexaploids.

Typical parental genotype	Number of genotypes in genus	Frequencies of gene-combinations in the gametic output				÷
		AB	*Ab*	*ab*	*aB*	
AB/ab/ab/ab/ab/ab	4	1 − y	y	5 − y	y	6
Ab/aB/ab/ab/ab/ab	4	y	5 − y	20 + y	5 − y	30
AB/Ab/ab/ab/ab/ab	4 + 4	5 − 4y	5 + 4y	20 − 4y	4y	30
Ab/Ab/aB/ab/ab/ab	4 + 4	2y	10 − 2y	15 + 2y	5 − 2y	30
AB/Ab/Ab/ab/ab/ab	4 + 4	5 − 3y	10 + 3y	15 − 3y	3y	30
AB/AB/ab/ab/ab/ab	4	5 − 4y	4y	10 − 4y	4y	15
AB/Ab/aB/ab/ab/ab	4	5 − 2y	5 + 2y	15 − 2y	5 + 2y	30
Ab/Ab/aB/aB/ab/ab	4	2y	5 − 2y	5 + 2y	5 − 2y	15
AB/AB/Ab/ab/ab/ab	4 + 4	10 − 6y	5 + 6y	15 − 6y	6y	30
AB/Ab/Ab/aB/ab/ab	2 + 2	1	2	2	1	6
AB/AB/AB/ab/ab/ab	2	5 − 3y	3y	5 − 3y	3y	10
AB/AB/Ab/aB/ab/ab	2	10 − 3y	5 + 3y	10 − 3y	5 + 3y	30

As before, we suppose that there are two alleles available at both loci. These will be denoted by A,a and B,b. Using y for the recombination fraction between the loci, the frequencies of the various gene-combinations in the gametic output are shown in table 3 for one representative genotype from each of the twelve genera. Comparing this with table 2, we see that corresponding with the unique neutral tetrasomic genotype, namely biduplex neutral, there are four neutral hexasomic genotypes. These are all duplo-triplex and comprise a conjugate pair of isomorphic sets, each of two genotypes. From table 3, in a parallel manner to that outlined previously for tetrasomic inheritance, we can arrive at the following relation which is analogous to (10).

$$\begin{aligned} p_{n+1}(AB) = p_n(AB) + (y/15)\,[&9p_n(Ab)p_n(aB) - 9p_n(AB)p_n(ab) \\ &+ 2p_n(Ab/Ab/aB) - 2p_n(AB/ab/ab) \\ &+ 2p_n(Ab/aB/aB) - 2p_n(AB/AB/ab) \\ &+ p_n(AB/Ab/aB) - p_n(AB/aB/ab) \\ &+ p_n(Ab/aB/ab) - p_n(AB/Ab/ab)\,]. \end{aligned} \tag{17}$$

From the gametic matrices for digenic hexasomic genotypes with two linked loci (BENNETT 1953) it can be shown that

$$\begin{aligned} y\,[&2p_n(Ab/Ab/aB) - 2p_n(AB/ab/ab) + 2p_n(Ab/aB/aB) - 2p_n(AB/AB/ab) \\ &+ p_n(AB/Ab/aB) - p_n(AB/aB/ab) + p_n(Ab/aB/ab) - p_n(AB/Ab/ab)\,] \\ &= 15(\gamma' + \delta')\,[p_n(AB) - p_{n-1}(AB)] \end{aligned} \tag{18}$$

where $\gamma' = 3y/5$ and

$$\begin{aligned} 15(\gamma' + \delta') = &6f_1 + 4f_2 + 4f_3 + 2f_4 + 2f_5 + 2f_6 + 2f_7 + f_9 - f_{10} \\ &- 2f_{11} - 2f_{14} - f_{16} - 4f_{17} - f_{18} - 2f_{19} - 3f_{20} \\ &+ 3f_{21} + 3f_{22} + 2f_{23} + 2f_{24} + f_{25} + f_{26} + f_{27} + f_{28} \\ &- f_{33} - f_{34} - 2f_{35} - 2f_{36} - 3f_{37} - 3f_{38} - 2f_{39} - 2f_{40}. \end{aligned} \tag{19}$$

Equation (18) is the analogue of (12). It follows that

$$\begin{aligned} p_{n+1}(AB) = p_n(AB) + (\gamma' + \delta')\,[p_n(AB) - p_{n-1}(AB)] \\ + \gamma'[p(A)p(B) - p_n(AB) \end{aligned} \tag{20}$$

This is of the same form as equation (13). Hence the analysis for two loci showing hexasomic inheritance may be derived from that given earlier in this paper for two tetrasomic loci on replacing γ and δ by γ' and δ' respectively. In particular, it follows that at equilibrium, two hexasomic factors are associated at random, the frequencies being the products of the equilibrium frequencies for the separate loci.

SUMMARY

Random mating with linked factors is considered in a population of organisms which exhibit tetrasomic or hexasomic inheritance.

Tables are presented giving gene-combination frequencies in the gametic

output in terms of the recombination fraction for a pair of linked loci. Recurrence relations are established for these frequencies and solved quite generally.

It is shown that at equilibrium, linked factors are associated at random.

LITERATURE CITED

BENNETT, J. H., 1949 M.A. thesis, University of Melbourne.

1953 Linkage in hexasomic inheritance. Heredity **7:** 265–283.

1954 On the theory of random mating. Ann. Eug. **18:** 311–317.

FISHER, R. A., 1947 The theory of linkage in polysomic inheritance. Phil. Trans. Roy. Soc. B **233:** 55–87.

1949 The Theory of Inbreeding. Oliver and Boyd.

FISHER, R. A., and K. MATHER, 1943 The inheritance of style length in *Lythrum salicaria*. Ann. Eug. **12:** 1–23.

GEIRINGER, H., 1949 Chromatid segregation in tetraploids and hexaploids. Genetics **34:** 665–684.

HALDANE, J. B. S., 1930 Theoretical genetics of autopolyploids. J. Genet. **22:** 359–372.

HARDY, G. H., 1908 Mendelian proportions in a mixed population. Science **28:** 49–50.

THE ASSOCIATION OF TWO GENETIC LINKAGE GROUPS IN BARLEY WITH ONE CHROMOSOME [1]

H. H. KRAMER, ROSEMARIE VEYL AND W. D. HANSON
Purdue Agricultural Experiment Station, Lafayette, Indiana

Received July 1, 1953

BARLEY, with seven chromosome pairs, has had genes assigned to seven linkage groups. ROBERTSON *et al.* (1941, 1947) and SMITH (1951) have so completely summarized the linkage studies on which these groups are based that further review is unnecessary here. None of the linkage groups has yet been associated with a specific chromosome pair, as has been done in maize, nor has it been definitely shown that a different chromosome pair is associated with each linkage group. Independent assortment of genes in one linkage group from those in another is still possible, even though on the same chromosome, if there is a long unmapped region between them.

The purpose of this paper is to present evidence from a cytogenic study of nine chromosomal interchanges in barley, which indicates that two linkage groups, III and VII, may be associated with the same chromosome, and that a different chromosome is associated with each of the other five linkage groups.

WHITE and BURNHAM (1948) and BURNHAM (1951) summarized the pairing relationships in crosses among five of these interchange lines and showed that six different chromosomes, which they designated by the letters a to f, were involved. HANSON and KRAMER (1949, 1950) and HANSON (1952) presented linkage data which identified the two linkage groups associated with each of three of these five lines. HAGBERG and TJIO (1952), also working with several interchange lines furnished by BURNHAM, were able to identify the chromosomes involved in two lines by comparing idiograms of homozygous interchanges with those of normal stocks. Though these two lines have not been used in the studies presented here, their work will permit assigning several of the linkage groups to specific morphologically identifiable chromosomes.

MATERIALS AND METHODS

Of the nine interchange lines used, seven (five of which were homozygous for the interchange) were kindly supplied by C. R. BURNHAM, University of Minnesota. They were produced by X-ray treatment of seed of the variety Mars. The pedigrees of the lines, the chromosomes involved in five as summarized by BURNHAM (1951) and the linkage groups involved in three (HANSON 1952) are presented in table 1; for convenience in presentation, designations 1 to 9 as shown in the first column will be used throughout this paper.

[1] Published as Journal Paper No. 702 of the Purdue University Agricultural Experiment Station.

TABLE 1

Interchange, origin and known chromosomes and linkage groups involved.

Interchange no.	Acc. or 1950 cult.	Minnesota culture	Chromosome involved[1]	Linkage group[2]
1	A272	C1025	....	...
2	A273	C1478	....	...
3	A298	C1385	a + b	....
4	A299	C1405	c + d	....
5	A300	C1420	e + f	I + IV
6	A301	C1432	c + e	IV + VI
7	A302	C1456	a + e	II + IV
8	C156	....	....	....
9	C160	....	....	....

[1]Burnham (1951).
[2]Hanson (1952).

To obtain genetic linkage data, each of the interchange lines was crossed with each of several standard normal linkage testers differentiated by contrasting characters controlled by factor pairs in each of the linkage groups. The characters used, their symbols, the linkage group to which each is assigned, the genotype of the interchange lines and the genetic tester lines having the contrasting characters are summarized in table 2. Since some of the genetic tester stocks were heterozygous for seedling lethal marker genes, F_2

TABLE 2

Linkage groups, marker genes used, the interchange genotype and genetic tester stocks used in linkage tests with interchanges in barley.

Linkage group	Character	Gene symbol	Genotype of interchanges	Genetic tester used
I	Two vs. six-row	*V, v*	*vv*	293
II	Black vs. white pericarp + lemma	*B, b*	*bb*	293
III	Covered vs. naked caryopsis	*N, n*	*NN*	293, 7
IV	Hooded vs. awned glume	*K, k*	*kk*	293, 4, 5
	Green vs. light green seedling	Lg_3, lg_3	Lg_3Lg_3	293 seg.
	Blue vs. non-blue aleurone	*Bl, bl*	*blbl*	296
V	Long vs. short-haired rachilla	*S, s*	*SS*	294, 5, 6
	Rough vs. smooth awn	*R, r*	*rr*	294, 5, 6, 7
VI	Green vs. xantha seedling	*Xc, xc*	*XcXc*	294 seg.
VII	Green vs. virescent seedling	*Yc, yc*	*YcYc*	296 seg.
	Starchy vs. waxy endosperm	*Wx, wx*	*WxWx*	297

seeds from each F_1 plant were space-planted in separate rows to differentiate populations segregating for lethals. Such populations are presented separately only when linkage with a lethal seedling marker gene was found. F_2 plants were classified for spike fertility and for the marker genes. F_3 plant rows were grown from F_2 plants of selected phenotypes in most crosses but only F_3 data which indicate linkage or which are derived from F_2 data which indicate linkage are presented.

Tests for linkage were made using chi square tests for independence cal-

culated from formulas derived from those given in table 4 of HANSON and KRAMER (1950). For the four class segregation the formula used was

$$\chi^2 = (b + 3c - a - 3d)^2/3N, \text{ D.F.} = 1,$$

and for the two class segregations due to the elimination of recessive seedling lethals,

$$\chi^2 = (b - a)^2/N, \text{ D.F.} = 1,$$

which is in effect a test of significance for the deviation from a 1 : 1 ratio. When the deviations from a 1 : 1 ratio were significant, 2×2 contingency tables were used for the sterility and marker gene classifications and chi square for independence calculated in the usual manner.

For F_3 data χ^2 tests were calculated according to the formulae

$$\chi^2 = (2e - f)^2/2N, \text{ D.F.} = 1,$$

and

$$\chi^2 = (h - 2g)^2/2N, \text{ D.F.} = 1,$$

for the genotypes of partially sterile *A* and fully fertile *A* F_2 plants respectively.

Normal plants from non-homozygous interchange lines which gave semi-sterile F_1 plants in crosses with the genetic testers, were selected to establish the homozygous interchange lines. The nine homozygous interchange lines were then crossed in all possible combinations and sporocytes of the F_1 plants examined cytologically. Spikes were killed and fixed in Carnoy's solution and stored in a refrigerator in 80% alcohol. Aceto-carmine smears were examined at metaphase I, or when possible at diakinesis.

RESULTS OF GENETIC STUDIES

For the interchange lines not previously reported, summaries of the F_2 segregation for partially sterile vs. fully fertile spikes (S vs. F) and for each of the marker gene pairs used to test for linkage are given in tables 3–7. From table 3, linkage of partial sterility with *Nn* in linkage group III and with *Ycyc* in group VII was found. Since *Wxwx*, also in group VII might be expected to show linkage, further tests in F_3 were made of the 113 *SWx* and the 117 *FWx* plants from cross 1×297 and the results are shown in the first line of table 8. No evidence for linkage was found. If group VII is involved the point of interchange must be nearer to *Ycyc* than to *Wxwx*.

In table 4, sterility due to interchange 2 appears to be linked with *Nn* and with *Ss* and *Rr* in group V. In view of the cytological results presented later the possibility of linkage with *Wxwx* in VII also should be examined. The F_2 data failed to show significant deviation from independence of *Wxwx* and the sterility classification but is in the expected direction if linkage were present. In F_3 (line 2, table 8) the genotypes of the 93 *FWx* F_2 plants from cross 2×297 indicate linkage. Thus groups III, V, and possibly VII are involved in this interchange.

TABLE 3

Summary of F_2 segregation in crosses between barley interchange no. 1 and linkage testers.

Cross	Gene pair	Phenotype and class				Total N	χ^2 for independence
		SA a	FA b	Sa c	Fa d		
1 × 293	*Vv*	127	84	30	33	294	3.07
1 × 293	*Bb*	128	103	29	34	294	1.81
1 × 293, 7	*Nn*	258	196	39	91	584	27.13[2]
1 × 293, 4, 5	*Kk*	388	316	112	120	936	3.28
1 × 293	Lg_3lg_3	48	35	....	....	83	2.03
1 × 296	*Blbl*	135	93	39	34	301	0.81
1 × 294, 5, 6	*Ss*	396	315	121	111	943	0.92
1 × 296	*Rr*	45	50	17	16	128	0.17
1 × 296[1]	*Rr*	83	48	29	13	173	0.45
1 × 294	*Xcxc*	12	11	....	....	23	0.04
1 × 296	*Ycyc*	112	61	....	....	173	15.01[2]
1 × 297	*Wxwx*	113	117	27	33	290	0.23

[1]Segregating for *Ycyc*.
[2]P <.01.

The linkage relationships of interchange 3 are calculated in table 5. Linkage of sterility with *Nn*, *Blbl* and *Ycyc* are indicated. Linkage with *Blbl* in group IV was not substantiated by linkage with Lg_3lg_3, also in IV, nor with *Kk* in spite of a very large population. In F_3 (table 8), only one out of four populations substantiated linkage with *Blbl* and non-linkage with Lg_3lg_3 was verified.

The segregation in F_3 of the *SK* phenotype from the cross of 3 × 293 gives a significant chi square value but the deviation is in a direction opposite to that expected from linkage. Linkage with *Ycyc* was verified in one F_3 population and no linkage with *Wxwx* was found either in F_2 or F_3. On the basis of genetic data above, chromosomes carrying linkage groups III and possibly VII may be involved in interchange 3. Association with IV appears doubtful.

TABLE 4

Summary of F_2 segregation in crosses between interchange no. 2 and linkage testers.

Cross	Gene pair	Phenotype and class				Total N	χ^2 for independence
		SA a	FA b	Sa c	Fa d		
2 × 293	*Vv*	19	14	11	4	48	1.77
2 × 293	*Bb*	21	13	9	5	48	0.11
2 × 293, 7	*Nn*	140	70	33	73	316	38.08[2]
2 × 293, 4, 5	*Kk*	205	148	75	48	476	0.40
2 × 293	Lg_3lg_3	30	18	....	....	48	3.00
2 × 296	*Blbl*	52	51	15	17	135	0.12
2 × 294, 5, 6	*Ss*	265	116	52	130	563	86.85[2]
2 × 294, 5, 6	*Rr*	114	85	15	23	237	3.95[1]
2 × 297	*Wxwx*	117	93	26	32	268	2.19

[1]P <.05.
[2]P <.01.

TABLE 5

Summary of F_2 segregation in crosses between interchange no. 3 and linkage testers.

Cross	Gene pair	Phenotype and class SA a	FA b	Sa c	Fa d	Total N	χ^2 for independence
3 × 293	*Vv*	94	72	33	21	220	0.30
3 × 293	*Bb*	87	70	40	23	220	1.75
3 × 293, 7	*Nn*	190	83	12	87	372	98.77[3]
3 × 293, 4, 5	*Kk*	332	268	117	106	823	0.39
3 × 293	Lg_3lg_3	36	23	....	....	59	2.86
3 × 296	*Blbl*	76	46	14	30	166	12.22[3]
3 × 296[1]	*Blbl*	105	58	18	35	216	15.13[3]
3 × 294, 5	*Ss*	262	224	96	80	662	0.05
3 × 296	*Rr*	65	64	25	12	166	2.90
3 × 296[1]	*Rr*	95	70	28	23	216	0.11
3 × 296	*Ycyc*	123	93	....	....	216	4.17[2]
3 × 297	*Wxwx*	62	62	13	15	152	0.08

[1]Segregating for *Ycyc*.
[2]P <.05.
[3]P <.01.

Linkage of any of the marker genes and sterility due to interchange 4 was difficult to establish. Only *Xcxc* in linkage group VI showed indication of linkage (table 6) which was verified in F_3 (table 8).

Data on lines 8 and 9 was limited to the F_2 results in table 7. Sterility for line 8 shows linkage with *Nn* in III and *Wxwx* in VII while that due to line 9 shows association with *Nn*.

CYTOLOGICAL OBSERVATIONS

Cytological observations were obtained on 32 of the 36 possible F_1 hybrids between the 9 interchange lines (table 9). With one exception these could be

TABLE 6

Summary of F_2 segregation in crosses between interchange no. 4 and linkage testers.

Cross	Gene pair	Phenotype and class SA a	FA b	Sa c	Fa d	Total N	χ^2 for independence
4 × 293	*Vv*	102	99	32	25	258	0.42
4 × 293	*Bb*	108	92	26	32	258	1.49
4 × 293, 7	*Nn*	239	238	60	60	597	0.00
4 × 293, 4, 5	*Kk*	330	313	124	113	880	0.10
4 × 293	Lg_3lg_3	71	62	....	....	133	0.61
4 × 296	*Blbl*	128	116	21	28	293	1.24
4 × 294, 5	*Ss*	241	225	79	77	622	0.05
4 × 296	*Rr*	119	115	30	29	293	0.00
4 × 294	*Xcxc*	26	12	....	....	38	5.16[1]
4 × 296	*Ycyc*	109	109	....	....	218	0.00

[1]P less than .05.

TABLE 7

Summary of F_2 segregation in crosses of interchanges 8 and 9 with linkage testers.

Cross	Gene pair	Phenotype and class				Total N	χ^2 for independence
		SA a	FA b	Sa c	Fa d		
8 × 297	*Nn*	55	40	2	25	122	19.28[2]
8 × 294	*Kk*	88	59	23	26	196	2.45
8 × 294	*Ss*	81	70	30	15	196	1.97
8 × 294, 7	*Rr*	60	73	20	13	166	2.32
8 × 297	*Wxwx*	46	42	11	23	122	4.37[1]
9 × 297	*Nn*	26	11	3	13	53	12.74[2]
9 × 294, 7	*Rr*	26	21	6	5	58	0.02
9 × 297	*Wxwx*	20	17	9	7	53	0.06

[1]P <.05.
[2]P <.01.

characterized at MI either by a ring of six chromosomes and four pairs, or by two rings of four and three pairs. The ⊙6 indicates that one chromosome pair is common to both interchanges while 2⊙4 indicates that two different pairs are involved. CALDECOTT and SMITH (1952, figs. 6–8) have illustrated these configurations.

All intercrosses involving lines 3, 4 and 5 show 2⊙4 and agree with the results presented by BURNHAM (1951) showing that 6 different chromosomes are involved in interchanges in these three lines. Intercrosses between lines 5, 6 and 7 show that one chromosome involved is common to interchanges in all three lines. BURNHAM has designated this e and HANSON (1952) has shown that each of the three lines shows linkage with genes in group IV. The ⊙6 in the F_1 of 3 × 7 indicates a chromosome in common to be involved in

TABLE 8

Summary of F_2 genotypes of selected F_2 phenotypes as determined by breeding behavior in F_3.

Cross	Population[1]	Gene pair	Genotype		Total N	χ^2	Genotype		Total N	χ^2
			SAA e	SAa f			FAA g	FAa h		
1 × 297	A	*Wxwx*	37	76	113	0.02	44	73	117	0.96
2 × 297	A	*Wxwx*	44	73	117	0.96	42	51	93	5.85[2]
3 × 293	A	*Kk*	17	50	67	1.91	7	37	44	6.01[2]
3 × 295	A	"	20	62	82	2.95	21	33	54	.75
3 × 293	B	Lg_3lg_3	14	22	36	0.50	8	15	23	0.02
3 × 296	A	*Blbl*	23	53	76	0.32	16	30	46	0.04
3 × 296	B	"	23	82	105	7.17[2]	23	35	58	1.04
3 × 296	B	*Ycyc*	38	83	121	0.20	41	50	91	5.63[2]
3 × 297	A	*Wxwx*	25	37	62	1.36	22	40	62	0.13
4 × 294	B	*Xcxc*	1	25	26	10.17[3]	10	2	12	13.50[3]

[1]A = from populations not segregating for lethal seedling, B = segregating.
[2]P less than .05.
[3]P less than .01.

TABLE 9

Summary of cytological configurations observed in F_1 hybrids among 9 homozygous interchange lines in barley and the chromosomes involved.

Interchange	1	2	3	4	5	6	7	8	9
1	Tb – d								
2	1C4, 5 II[1]	Tb – d							
3	1⊙6, 4 II	1⊙6, 4 II	Ta – b						
4	1⊙6, 4 II	1⊙6, 4 II	2⊙4, 3 II	Tc – d					
5	....	2⊙4, 3 II	2⊙4, 3 II	2⊙4, 3 II	Te – f				
6	2⊙4, 3 II	2⊙4, 3 II	2⊙4, 3 II	1⊙6, 4 II	1⊙6, 4 II	Tc – e			
7	2⊙4, 3 II	2⊙4, 3 II	1⊙6, 4 II	2⊙4, 3 II	1⊙6, 4 II	1⊙6, 4 II	Ta – e		
8	1⊙6, 4 II	1⊙6, 4 II	1⊙6, 4 II	1⊙6, 4 II	2⊙4, 3 II	1⊙6, 4 II	....	Tb – c	
9	1⊙6, 4 II	....	....	2⊙4, 3 II	1⊙6, 4 II	2⊙4, 3 II	2⊙4, 3 II	1⊙6, 4 II	Tb – f

[1]Mostly chains of 4 chromosomes, occasionally a ring, infrequently 7 II.

both lines (designated a by BURNHAM). A similar conclusion may be reached regarding the F_1 of 4×6 and this chromosome has been designated c. Using these five lines as reference points the chromosomes involved in the remaining four lines are easily established and are shown in the diagonal cells in table 9. That lines 1 and 2 involve the same two chromosomes is verified by the fact that five and occasionally 7 pairs are observed in the F_1 between them.

DISCUSSION

The genetic data in tables 3–8, and the published data of HANSON and KRAMER (1949, 1950) and HANSON (1952) indicate that genes in each of the seven linkage groups have shown associations with sterility in crosses of linkage testers to one or more of the 9 interchange lines. It is noteworthy, however, that in four of the five cases in which *Nn* in group III showed association with sterility, either *Ycyc* or *Wxwx* in group VII also gave evidence of association with sterility in the same interchange.

TABLE 10

A summary of possible linkage groups involved from genetic studies and the chromosomes involved from cytological observations in 9 interchange lines.

Interchange	Possible linkage group	Chromosome involved	Suggested linkage group
1	III, VII	b – d	III, VII – V
2	III, V, VII	b – d	III, VII – V
3	III, IV, VII	a – b	II – III, VII
4	VI	c – d	VI – V
5	I, IV	e – f	IV – I
6	IV, VI	c – e	VI – IV
7	II, IV	a – e	II – IV
8	III, VII	c – b	VI – III, VII
9	III	b – f	III, VII – I

The cytological evidence indicates that only six chromosomes are involved in interchanges in the 9 lines. The genetic and cytological data are summarized for comparison in table 10. There appears to be little question that chromosome b carries linkage group III and e carries IV. Linkage of group IV appears not to be involved in line 3 in spite of a significant χ^2 test for *Blbl. Lg*$_3$*lg*$_3$ and *Kk* in the same group showed no linkage, F_3 data were not conclusive, and the cytological data rule out this group. Chromosome a involved in lines 3 and 6 probably carries linkage group II. Chromosome c and group VI are associated in lines 4 and 6, and in line 8, linkage with group VI was not tested. Chromosome f is involved in lines 5 and 9 but linkage data with group I are available only for line 5. Chromosome d must be associated with groups V or VII. Assigning group VII to d, however, is inconsistent with the cytological data on lines 3 and 8 and with the genetic data for line 4. Assigning group V to chromosome d causes no inconsistency either in the cytological or the genetic data though linkage was undetected in lines 1 and 4. The conclusion that both linkage groups III and VII are carried by chromo-

some b appears warranted, and the other five groups must be distributed among 5 different chromosomes. The suggested linkage groups are summarized in the last column of table 10.

HAGBERG and TJIO (1950) have presented a chromosome idiogram for barley and have numbered the five non-satellite chromosomes from I to V in order of falling total length, I being longest. Chromosome VI was the smaller satellite chromosome with a large satellite and VII was the longer chromosome with a small satellite. Later (1952), using five interchanges from BURNHAM which included lines 3, 5 and 6 studied here they attempted to determine the chromosomes involved in relation to their standard idiogram. Unfortunately the chromosomes involved in the three lines studied here could not be identified. However, on two other interchanges involving b + d and b + (g) +, they were able to show that chromosome b is III, the third longest non-satellite chromosome, d is VII and g is VI. It would appear then, that these three chromosomes carry linkage groups III and VII, V, and the last unestablished linkage group in barley, respectively.

SUMMARY AND CONCLUSIONS

Nine reciprocal chromosomal interchange lines of barley were tested for linkage of the interchange points with one or more factor pairs in each of the seven linkage groups in barley.

The nine interchanges also were intercrossed in all combinations and pairing relationships observed in the F_1 hybrids.

In correlating the genetic linkage data with the cytological observations the conclusion was reached that two linkage groups, heretofore designated III and VII are carried by the same chromosome and of the remaining five linkage groups, each is on a different chromosome.

In relating these results to the chromosome idiograms of HAGBERG and TJIO (1952) it appears probable that linkage groups III and VII are carried by the third longest non-satellite chromosome, the larger of the two satellite chromosomes probably carries linkage group V, while the smaller of the satellite chromosomes probably carries a linkage group to which no genes have as yet been assigned.

ACKNOWLEDGMENT

The authors wish to acknowledge the grant-in-aid of the Purdue Research Foundation which made many of the genetic studies possible.

LITERATURE CITED

BURNHAM, C. R., 1951 Barley workers' conference report. Mimeo.

CALDECOTT, R. L., and LUTHER SMITH, 1952 The influence of heat treatments on the injury and cytogenetic effects of X-rays on barley. Genetics **37**: 136–157.

HAGBERG, A., and J. H. TJIO, 1950 Cytological localization of the translocation point for the barley mutant erectoides 7. Hereditas **36**: 487–491.

1952 Cytological studies on some homozygous translocations in barley. Anales estac. exptl. Aula Dei **2**(3–4) : 215–223.

HANSON, W. D., 1952 An interpretation of the observed amount of recombination in interchange heterozygotes of barley. Genetics **37:** 90–100.

HANSON, W. D., and H. H. KRAMER, 1949 The genetic analysis of two chromosome interchanges in barley from F_2 data. Genetics **34:** 687–700.

1950 The determination of linkage intensities from F_2 and F_3 data involving chromosomal interchanges in barley. Genetics **35:** 559–569.

KRAMER, H. H., 1953 Recombination in selfed chromosome interchange heterozygotes. In press. Iowa State College Press.

ROBERTSON, D. W., G. A. WIEBE and F. R. IMMER, 1941 A summary of linkage studies in barley. J. Amer. Soc. Agron. **33:** 47–64.

ROBERTSON, D. W., G. A. WIEBE and R. G. SHANDS, 1947 A summer of linkage studies in barley: Supplement 1, 1940–1946. J. Amer. Soc. Agron. **39:** 464–473.

SMITH, L., 1951 Cytology and genetics of barley. Bot. Rev. **17:** 1–51, 133–202, 285–355.

WHITE, F. H., and C. R. BURNHAM, 1948 Translocations in barley. Abstract of paper presented at the annual meeting of the American Society of Agronomy. Mimeo.

RECOMBINATION IN PHAGE T2: ITS RELATIONSHIP TO HETEROZYGOSIS AND GROWTH

CYRUS LEVINTHAL [1]

Department of Physics, University of Michigan, Ann Arbor, Michigan

Received July 8, 1953

WHEN genetic recombination was discovered in bacteriophage (HERSHEY and ROTMAN 1948) it was natural to think of the process in the same way that one thought of recombination in higher organisms. This type of approach has met with varying degrees of success. Linkage groups have been established with roughly additive map distances between markers and three-factor crosses can be understood in terms of double cross-overs. However, there is a considerable body of evidence on the increasing recombinant frequency with time after infection and on three-parent crosses (see DOERMANN 1953) which makes it impossible to understand a phage cross (i.e., the output of a single, plurally infected bacterium) in terms of a single mating event.

The repeated mating theory of VISCONTI and DELBRUCK (1953) explains all of the accumulated data on phage genetics except those involving the phenomenon of heterozygosis (HERSHEY and CHASE 1951; HERSHEY 1953b). In this theory, a mixedly infected bacterium is considered as a population mixture of randomly mating particles, which are in a noninfective state designated as vegetative. The infected cell is assumed to have a pool of vegetative phage in which there is a continual approach to genetic equilibrium by successive matings. This theory has the advantage of introducing no postulates about the basic mechanism of recombinant production that have not already been used in the genetics of higher organisms.

The experiments and calculations reported here arose from consideration of models of recombination in phage in which the production of recombinants is treated as a special case of vegetative phage replication. The requirement that the mechanisms be similar to those which seem to apply in higher organisms was relaxed. But it is of course necessary that any new theory make the same predictions as that of VISCONTI and DELBRUCK where the latter have successfully explained the experimental data.

This paper will be divided into three parts; first, the results of experiments designed to investigate the nature of the heterozygotes; second, calculations which indicate that heterozygote formation provides a mechanism for the production of all of the observed recombinants and, third, speculations on the implications of those findings for the theory of recombination and replication in phage.

[1] This study was supported by a grant from the National Microbiological Institute of the National Institutes of Health, U. S. Public Health Service.

REVIEW OF PREVIOUS DATA ON HETEROZYGOTES

The data of HERSHEY and CHASE (1951) can be summarized as follows:

1) A heterozygote is defined as a free virus particle that produces two types of progeny when it infects a sensitive bacterial cell. In crosses of r^+ by r (rapid lysis mutant) they are scored by the mottled plaques which they form; these contain a mixture of r^+ and r particles. Heterozygous particles recognized by this mixed progeny will be called mottlers.

2) The five different markers tested in crosses $r \times r^+$ all produced about 2% heterozygotes recognizable as mottlers. A cross $h^+ \times h$ (host range mutant) also produced 2% plaques on sensitive bacteria which segregated to form h and h^+ progeny. Thus apparently any marker will be heterozygous in about 2% of the progeny of an equal input cross.

3) The frequency of the heterozygotes for a single marker is constant at 2% whether the cells are lysed prematurely, at the end of the normal latent period, or lysis is delayed. This is to be contrasted with the proportion recombinants which, for closely linked markers, increases by a factor of 4 to 6 when lysis is delayed (LEVINTHAL and VISCONTI 1953).

4) When the two markers are unlinked or distant, heterozygosis occurs almost independently in each; only about 3% of the heterozygotes show the effect for both markers.

5) When two markers are closely linked double heterozygosis is more frequent; for the markers h and r_{13} about 75% of the r mottlers are also heterozygous for h.

Two conclusions are suggested by these data. First, the heterozygous region of the chromosome is short. Its length is on the average greater than the $h\ r_{13}$ distance and less than the $h\ r_7$ distance. The rest of the chromosome may be homozygous diploid or hemizygous. Second, the heterozygotes are not reproduced as such inside the bacterial cell since if they did one would expect a cumulative increase in the fraction of the progeny showing heterozygosis as the burst size increases with delayed lysis. Also, the progeny of a heterozygous particle show only the same incidence of heterozygosis as do the progeny of mixedly infected bacteria.

Possible alternatives

Two conceptions of a partial duplication or partial diploidy of a haploid chromosome suppose (I) a small piece attached to the side of a normal chromosome or (II) partial overlapping of two pieces of different parental chromosomes, as shown in figure 1a. The equivalent alternatives with complete diploidy are shown in figure 1b. Either of those possibilities is consistent with the data of HERSHEY and CHASE, but they can be distinguished in a three-factor cross. On model I the markers on opposite sides of the duplication would be contributed by the same parent, and on model II they would be contributed by unlike parents. Thus in a three-factor cross ($A\ B\ C \times A^+\ B^+\ C^+$) heterozygotes at the B locus should be $A\ C$ or $A^+\ C^+$ on model I but $A\ C^+$ or $A^+\ C$ on model II.

There are, however, two obstacles to realizing this idealized experiment. 1) We do not yet have three different, separately recognizable markers on one linkage group in phage and 2) when the first mature intracellular phage appears many recombinants have already been formed, so even the first mature heterozygotes formed will not have been made by combining a pure population of $A\,B\,C$ and $A^+\,B^+\,C^+$. They will have been formed by the mixed population of parental type vegetative phage and recombinants.

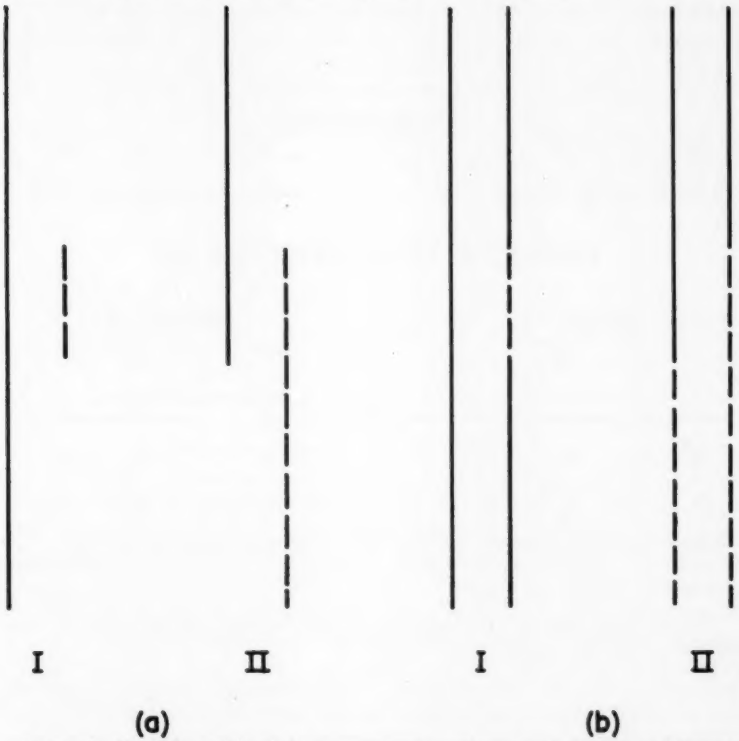

FIGURE 1.—Possible models of the heterozygotes consistent with the data of HERSHEY and CHASE. Solid lines represent the chromosomes from one parent and dotted those from the other parent.

The first difficulty is avoided by crossing $h\,r_2^+\,r_7 \times h^+\,r_2\,r_7^+$. These markers are on the same linkage group and in the order shown. In two-factor crosses the recombination frequency between h and r_2 is 16% and between r_2 and r_7 8%. But r_2 and r_7 are mutually epistatic, that is, a virus particle carrying any r factor will produce an r type plaque. Thus any mottler from this cross must contain both r_2^+ and r_7^+ as well as one of the r genes. The four possible segregation patterns for the r heterozygotes in this cross are 1)

$r_2^+ r_7^+/r_2 r_7^+$ (r_2 heterozygotes); 2) $r_2^+ r_7^+/r_2^+ r_7$ (r_7 heterozygotes); 3) $r_2 r_7^+/r_2^+ r_7$ and 4) $r_2^+ r_7^+/r_2 r_7$. Only the first two of these will lead to mottled plaques. The third will not produce mottling, as too few r^+ will be formed by recombination after plating. This was shown by HERSHEY and CHASE as well as in these experiments by the fact that bacteria infected with several r_2 and r_7 phage yield only a negligible number of mottlers when plated before burst. The fourth class can be ruled out by HERSHEY's finding that the mottled plaques in $h\,r \times h^+\,r^+$ crosses never segregated to form the two recombinants. We can now consider how the h character will be distributed in the two classes of mottlers on the two models for the heterozygotes shown in figure 1. It can be seen from figure 2 that mottlers of type 2), that is, r_7 het-

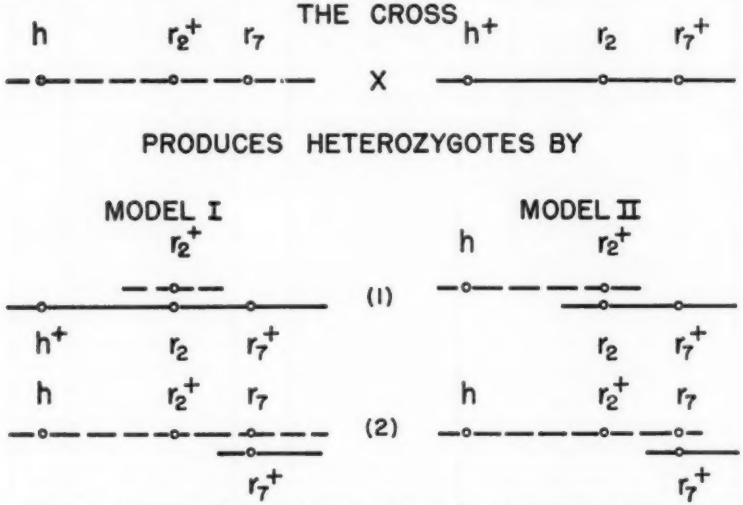

FIGURE 2.—Structure of the r_2 (above) and r_7 (below) heterozygotes from the cross $h\,r_7 \times r_2$ considering Models I and II of figure 1.

erozygotes, will have the h marker in either model, but the type 1) mottlers, that is r_2 heterozygotes, will have the h^+ character in model I and h in model II. Since Hershey showed that heterozygotes for r_2 are as frequent as those for r_7, we would expect on model I to find 50% of the mottled plaques containing h and on model II 100% containing h if there had been no recombination between the markers before the heterozygotes were formed.

We will now determine how these conclusions are modified by the fact that the vegetative pool contains recombinant particles as well as parentals. If we designate the total frequency of the recombinants between h and r_2 as m and the frequency between r_2 and r_7 as n, then to first order in m and n (that is considering only single crossovers) the frequency of parental genotypes in the population is $1-(m+n)$. In table 1 are listed the two parental and four

TABLE 1

Genotypes in the vegetative pool in a bacterium equally infected with hr_7 and r_2 phage. m is the recombinant frequency between h and r_2 and n is that between r_2 and r_7. All calculations assume m and n are small compared to 1.

Type	Frequency
A) $hr_2^+r_7$	½ (1 − (m + n))
B) $h^+r_2r_7^+$	½ (1 − (m + n))
C) $h^+r_2^+r_7$	½ m
D) $hr_2r_7^+$	½ m
E) $hr_2^+r_7^+$	½ n
F) $h^+r_2r_7$	½ n

Heterozygotes formed by "collisions" of above types, showing frequency and host range as calculated on model II.

Interacting types	Heterozygous for	h character	Frequency × 4 to first order in m and n
A × B	r_2	h	1 − 2(m − n)
A × D	r_2	h	m
B × C	r_2	h^+	m
B × E	r_2	h	n
B × E	r_2	h^+	n
A × B	r_7	h	1 − 2(m − n)
A × D	r_7	h	m
A × E	r_7	h	2n
B × C	r_7	h^+	m

Thus $hr_2r_2^+ = 1 - (m + n)$ and $hr_7r_7^+ = 1 - m$

recombinant types which could be produced by a single crossover or its equivalent, with their frequencies given in terms of the parameters m and n. If we consider only the results for premature or normal lysis, m and n are both small compared to one and we can neglect products of two such small quantities. In the lower part of table 1 are listed the results of such first order calculations for the frequency of *h* in the mottlers assuming only that the probability of a given type of pairing (or mating) is proportional to the product of the two population frequencies. The conclusion of these first order calculations is that the fraction of the r_2 mottlers showing *h* (designated $h\,r_2\,r_2^+$) is $[1 - (m + n)]$ and $h\,r_7\,r_7^+ = 1 - m$.

EXPERIMENTAL METHODS AND RESULTS

Crosses were made between $h\,r_2^+\,r_7$ and $h^+\,r_2\,r_7^+$ and the mottled plaques tested for their *h* character. If the majority were *h* it would indicate that the r_2 heterozygotes are recombinants between *h* and r_7.

All of the crosses were done using mutants of the phage T2H and the host bacterium *Escherichia coli* strain H. The plating was done with strains S and 2bc (S/T2) as the sensitive and resistant bacteria, respectively. All of these strains were originally obtained from HERSHEY and have been described by him (HERSHEY and ROTMAN 1949). The methods of adsorption and plating were those described by ADAMS (1950) as modified by VISCONTI and DEL-

BRUCK (1953) and LEVINTHAL and VISCONTI (1953). Premature lysis was produced by dilution into a chilled buffer containing M/100 KCN (DOERMANN 1952). In each cross the multiplicity of infection was measured by assaying for uninfected bacteria before burst. The infected bacteria were plated before burst to check for unadsorbed phage. The progeny of the crosses were assayed on strain S, sensitive to both h and h^+. On this indicator the average burst size and one class of recombinants, namely r^+, could be scored, and mottled plaques could easily be distinguished. All mottled plaques were counted and those not in contact with any other plaques on the plate were picked and suspended in buffer. The phage from each of the mottled plaques was then diluted and replated on mixed indicator plates with S and 2bc. These plates gave an additional check on the selection of mottlers since they showed both r^+ and r plaques; these plates were scored as to host range. For each cross

TABLE 2

Three-factor crosses.

Cross and condition	1 Input multiplicity	2 Average burst size	3 h in total progeny (percent)	4 r^+ in total progeny (percent)	5 Mottlers in total progeny (percent)	6 Mottlers with h observed (percent)	7 Mottlers with h expected (percent)	8 Mottlers with h expected (percent)
$hr_7 \times r_2$ premature lysis	8	1	47 ± 2.5	2.9 ± .2	1.3 ± .2	80 ± 2.7 226 tested	85	80
$hr_7 \times r_2$ normal lysis	7	100	45 ± 3.5	4.2 ± .4	1.5 ± .2	73 ± 4.2	79	72
$hr_7 \times r_2$ inhibited lysis	8	350	47 ± 2.5	14 ± 1	1.2 ± .2	57 ± 5	~55	54
$hr_2 \times r_7$ premature lysis	7	4	53 ± 2	3.0 ± .3	1.4 ± .2	29 ± 4	15	20

The fraction of h among the mottlers as calculated from model II is given in columns 7 and 8. The value expected from model I is given in column 3. No correction for negative interference has been made in column 7. The expected values corrected for negative interference (from experimental value of negative interference) are given in column 8.

about 120 mottled plaques were tested in this way and scored for host range, and the following data were thus obtained: 1) input multiplicity, 2) average burst size, 3) fraction of h in total progeny, 4) fraction r^+ in total progeny, 5) fraction of mottlers among the total progeny, and 6) fraction of h among the mottlers. The expected value of the fraction h among the mottlers was calculated for model II from 4) by making use of the fact that for these markers m = 2n and averaging $h\, r_2\, r_2^+$ and $h\, r_7\, r_7^+$. For model I the fraction of h among the mottlers would be equal to the fraction of h among the total progeny. The standard errors of the experimental data are calculated for sampling error alone. In the case of the percent h among the mottlers the sampling is believed to be unbiased since the scoring is unambiguous and the mottlers tested are picked from single indicator plates on which the host range character cannot be detected.

The results of three crosses $h\, r_7 \times r_2$ and one $h\, r_2 \times r_7$ are shown in table 2.

It can be seen that the results are in agreement with the expected values from model II and in disagreement with the expected values from model I. The significance of this agreement will be discussed below and we will now take up one further experiment.

It was assumed in the calculation of table 2 that the r_2 heterozygotes are as frequent as the r_7's. This point could be checked only by backcrossing r segregants from the mottlers with known r_2 and r_7 stocks.

The pooled phage from five r plaques from each mottler was back crossed with both r_7 and r_2 stocks. Standard procedures were used except that the inputs of the phage under test were only controlled to about a factor of three, and the final platings were done on plates crowded with r so that even a few r^+ plaques could be easily scored.

TABLE 3

Results of back crosses.

	Observed	Expected from	
		Model I	Model II
Percent r_2 heterozygotes among mottlers	42 ± 5%	50	50
Percent h among r_2 heterozygotes	75 ± 6	18	82
Percent h among r_7 heterozygotes	88 ± 4	88	88

The results of back crosses to determine the r character of 106 mottled plaques. The types obtained were as follows: 54 r_7h; 34 r_2h; 11 r_2h^+; 7 r_7h^+. The mottled plaques were obtained from the cross $hr_7 \times r_2$ premature lysis. No correction was made for the effect of negative interference.

The r phage from 106 mottled plaques in the progeny of the premature lysis $h\ r_7 \times r_2$ cross were tested by two back crosses each and the r character determined. Thus a true three-factor cross was done and the results so obtained are given in table 3. As can be seen, the results are in agreement with the expected values of model II.

INTERPRETATION OF THE EXPERIMENTS

The experimental results can be summarized as follows: 1) In a three-factor cross particles which are heterozygous for the middle marker, r_2, are generally recombinants for the end markers h and r_7, 2) as the lysis is delayed and the recombinant frequency increases, the fraction of the heterozygotes showing recombination for the end markers decreases, approaching 50% as genetic equilibrium is reached, 3) there is fair agreement between the observed percent h among the r mottlers and the value expected from the overlap model.

However, the problem of calculating the fraction of the heterozygotes created by parents which are themselves recombinants is the same as the problem of calculating the fraction of double crossovers among the class of particles having at least one crossover. VISCONTI and DELBRUCK (1953) investigated the apparent negative interference which one finds in this case. They pointed out that

in selecting the class with at least one crossover one is selecting phage which have been involved, on the average, in more mating processes. The calculations of this effect of inhomogeneity cannot be made accurately since there are unknown sources of variation among different bacterial cells. However, the correction can be made in our case using the experimental results on negative interference. These corrected values are shown in column 8 of table 2.

THE RELATIONSHIP OF HETEROZYGOSIS TO RECOMBINATION

We have demonstrated that a phage particle heterozygous in a certain region will produce progeny which are recombinants for markers on opposite sides of this region. But the question immediately arises as to whether these are the only intermediates that produce recombinants. There does not seem to be any way of answering this question rigorously but we can determine that this mechanism is a sufficient one. That is, we can calculate the number of recombinants which would be produced by the heterozygotes alone. As will be seen, they account, at least approximately, for the number of recombinants observed and by the principle of conservation of hypothesis we conclude that *all* recombinants in phage are produced from the heterozygotes.

We know from the fact that the types of heterozygotes produced change with time that new heterozygotes are continually being formed from whatever population exists in the vegetative pool. But from the fact that the percentage of heterozygotes does not increase with time, we conclude that the heterozygous condition is not reproduced as such. This assumption concerning the internal progeny of the heterozygotes is in agreement with findings of free phage. The progeny of a cross with two percent mottlers will have less than one mottler in a thousand after one cycle of growth under conditions of single infection in sensitive bacteria. Thus we conclude that heterozygosis occurs for any marker in two percent of all new vegetative particles in an equally infected bacterium, but these heterozygotes produce progeny which are haploid (or homozygous) for the marker investigated.

We will introduce some parameters necessary to describe the overlapping partial diploids. We will call the average length of the overlap piece L. The position of the overlap will be characterized by the distance, X, of its center from one end of the chromosome and we will call the probability that its center fall between X and $(X+dx)$, $P(X)dx$. That is $P(X)$ is the probability per unit length that the center of an overlap occurs at X. Using this notation we find that the probability of any part of an overlap region covering a particular gene is

$$\int_{-L/2}^{L/2} p(x)\, dx$$

but this is just the probability of finding heterozygosis in a particular marker which Hershey found to be two percent for all markers tested. The above integral will be independent of X if $P(X)$ and L are constants and under these conditions the integration can be performed to yield the relationship

$$PL = 2\% \qquad (1)$$

Let D hr_{13} be the distance between the genes h and r_{13} measured in the same units as L and X. Then the ratio of L to Dhr_{13} can be calculated from the fraction of the r_{13} mottlers which are also heterozygous in h, which HERSHEY measured to be 75%. Since the range of X which will produce double heterozygotes is (L – D) out of a total range L which will put r_{13} on the overlap we have:

$$L = 4\,D hr_{13} \tag{2}$$

If we consider a population in the vegetative pool consisting entirely of parental types, then recombinants will be produced between any two genes if they are separated by the overlap. Let R be the number of recombinants and N the total number of particles in the pool. Then

$$\frac{dR}{dN} = PD \tag{3}$$

where D is the distance between the genes. This expression correctly takes into account the end effects of structures like those shown in figure 3 if we assume that the progeny of the heterozygotes in the pool are distributed in the same way as to genetic type as are the progeny of the heterozygous free phage. This means that a structure like the one in figure 3 is counted as half of a recombinant for h and r_{13} since half of its progeny will be recombinants while it is counted as a full recombinant for h and r_1.

When the population in the pool has both parental types and recombinants we must modify (3) to take into account the fact that the process which makes a recombinant heterozygote from two parental types will make a parental type from two recombinants. Thus only the "collisions" between parental types will produce recombinants and the number of these is proportional to $(N - R)^2$ while the number of "collisions" between recombinants which can lead to the production of parents will be proportional to R^2. "Collisions" between one recombinant and one parental type cannot change the genetic distribution. We must also consider that the recombinants are able to reproduce their own kind and presumably at the same rate as the total population. Finally, equation (3) can be rewritten as

$$\frac{dR}{dN} = \frac{R}{N} + PD\,\frac{(N-R)^2}{N^2} - PD\,\frac{R^2}{N} = \frac{R}{N}(1 - 2PD) + PD \tag{4}$$

The solution to this equation for the recombinant frequency is

$$\frac{R}{N} = \frac{1}{2}\left[1 - \left(\frac{N}{N_0}\right)^{-2PD}\right]$$

where N_0 is the number of particles at infection when $R = 0$. If we write N/N_0 in terms of number of generations, and n, as $N = N_0 e^n$, then the solution becomes

$$\frac{R}{N} = \frac{1}{2}(1 - e^{-2PDn}) \tag{5}$$

This equation can be compared to equation (10b) of VISCONTI and DELBRUCK (1953) which for an equal input cross reads

$$\frac{R}{N} = \frac{1}{2}(1 - e^{-mP}) \tag{6}$$

where m is the number of rounds of random mating and P is the linkage. For unlinked markers P = ½ and the effective m for the normal cross must be 5 to account for the observed approach to genetic equilibrium. In our case heterozygotes could be formed only from some sort of a " collision " between opposite types, but half of the " collisions " will be identical particles. Furthermore, any model we have been able to construct would require the same assumption as that made by VISCONTI and DELBRUCK, namely only half of the

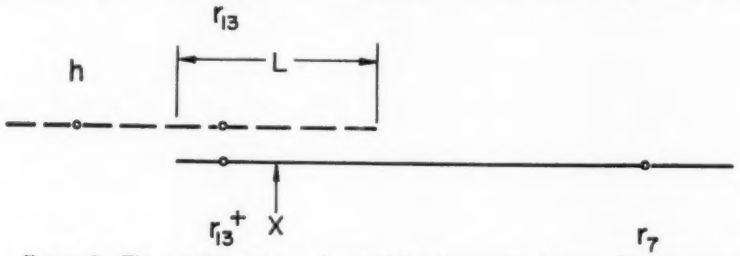

FIGURE 3.—The genetic structure of a particle heterozygous for r_{13}. The progeny of this heterozygote would be half $h\ r_{13}\ r_7$ and half $h\ r_{13}{}^+\ r_7$. That is, half of the progeny would be recombinants for h and r_{13} and all would be recombinants for h and r_7.

" collisions " between opposite types will yield recombinant type heterozygotes. Thus the maximum value of PD would be one-quarter and our equation (5) becomes identical to that of VISCONTI and DELBRUCK with m = n. From this we can conclude that the number of generations n in this treatment should have the same value as the number of rounds of mating m in the VISCONTI-DELBRUCK theory.

Now that we know the value of n for the normal cross and we know the value of PD for the $h\ r_{13}$ distance from equations (1) and (2), we can calculate the recombinants produced by this mechanism. From (1) and (2) we have PDhr_{13} = 0.5 percent, and with m = 5

$$\left(\frac{R}{N}\right)_{hr_{13}} = \frac{1}{2}(1 - e^{-.005 \times 5 \times 2}) = 2.5 \text{ percent}$$

which is to be compared to the value of 2.6 ± 0.3 percent observed in the normal cross (LEVINTHAL and VISCONTI 1953). Thus within the accuracy of the experiments the heterozygotes and their progeny account for the observed recombinants.

DISCUSSION

In the preceding calculations it has been shown that the heterozygotes are the intermediates in the production of recombinants in phage. These calcula-

tions did not require any detailed specification of a model, but before considering the models which are consistent with the findings, the assumptions used so far will be explicitly listed and discussed.

Assumptions used in the calculations

1) The basic ideas of the VISCONTI-DELBRUCK theory concerning population interaction are correct. That is, the phage interact (i.e., "mate" in a very general sense) in a pool of vegetative, noninfective particles within the bacterium. The " mating " is considered random both as to partner and time and there is a random and irreversible extraction from the pool to form intact nonmating phage particles. The justification of these hypotheses was discussed by VISCONTI and DELBRUCK and was based on the results of two- and three-factor crosses and on the gradual approach to genetic equilibrium with time.

2) The production of the heterozygotes involves some sort of an interaction between two unlike vegetative phages which, as seen from the experiments, leads to a particle with an overlapping structure.

3) The heterozygotes do not reproduce their own kind but produce progeny at the usual rate in the vegetative pool and the distribution as to type of these progeny is the same as that found for the heterozygous free phage. As stated above, this assumption is based on the constancy of the heterozygote frequency in the bursts after delayed lysis and its reduction after one cycle of growth in new bacteria.

4) The overlap segment is equally likely to occur at any point on the chromosome. This assumption is justified by HERSHEY's finding that the probability of heterozygosis is the same for the five markers tested. It is also assumed that the overlaps have a distribution in length such that few of them are shorter than one-quarter of the mean length. However, even if this were not true, it could only raise the number of recombinants from this mechanism by a small amount.

Implications of these findings for possible models

It has been very difficult to construct a model of mating in which two formed chromosomes come together to produce an overlapping heterozygote without correlating the mating with replication. The only scheme which seems possible is the one shown in figure 4 (a) and its equivalent in 4 (b). In this model we must assume that the unwanted ends are disposed of by an unknown process. It is not a very satisfactory model since it requires that unusually large amounts of chromosomal material be " thrown away," and that we introduce several other very artificial assumptions. For instance, unless we assume some special restriction we might expect to find structures of the type shown in figure 4 (c), but they would have been observed even if they had occurred in only a few percent of the heterozygotes. No simpler model seems to result from considering the heterozygotes as completely diploid.

Obviously the real motivation behind the use of this type of model is that it is similar to those which have been used successfully in higher organisms;

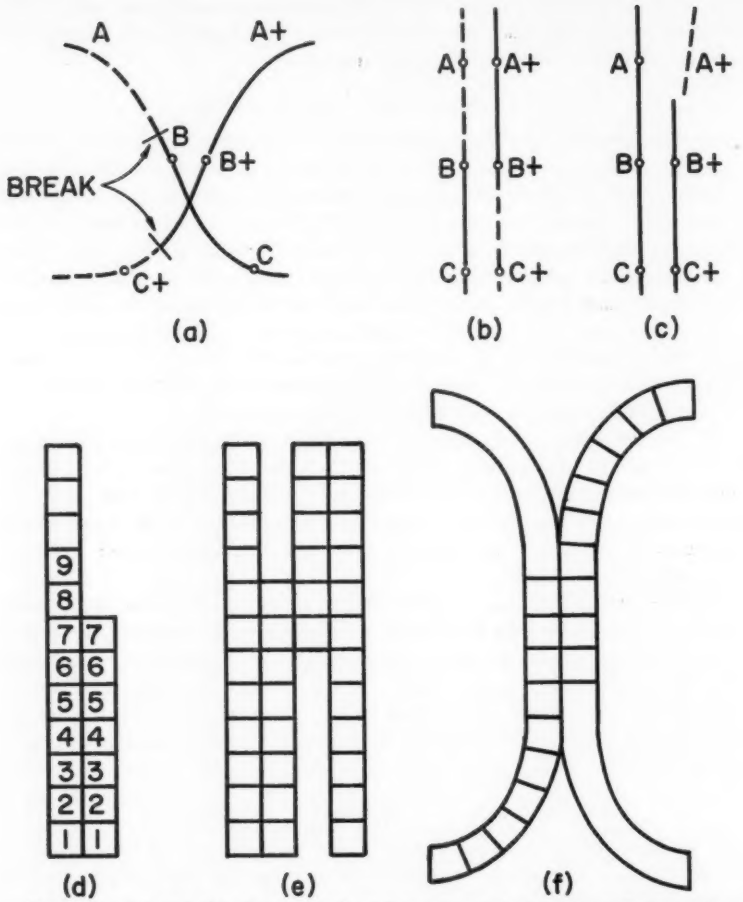

FIGURE 4.—(a) and (b) show methods of formation of the heterozygotes by breakage of formed chromosomes. The dotted portions must be thrown away. The type of structure shown in (c) must be ruled out by some new hypothesis. (d) shows the lateral zipper like model with the half completed daughter on left. (e) shows two parents on the outside and a newly formed heterozygote on the inside. (f) shows how the heterozygote (shown cross-hatched) could be formed above two paired parents. The daughters of the heterozygotes would be formed by replication on one side or the other.

but it is necessary to modify the usual concepts to such an extent that there does not seem to be any gain in simplicity by starting with the more usual mechanisms. No discussion has been given of models involving duplications in the usual sense, that is, linear insertions, since it does not seem possible to explain the segregation patterns in this way.

We will now consider the model which led to these experiments. In it we

assume that the recombinants, or in this case heterozygotes, are not constructed out of pieces of the previously formed chromosomes, but are formed *de novo* under the control of two previously completed structures.

We start with a model of replication involving lateral aggregation in a zipper-like fashion (see a suggestion by STURTEVANT quoted in HERSHEY and ROTMAN (1949) also BELLING (1933). This is pictured schematically in figure 4 (d) where one of the previously completed structures shown with solid lines is acting as a parent for a half-completed daughter. We assume that the structures consist of a linear array of sub-units and that the replication is the assembling of the sub-units in an order which is identical to that of the parent. We postulate some kind of an attachment between the end sub-unit and a free one which is identical to it, and then the attachment of a unit like the second, etc. If we think of the phage, it is likely that the problem of genetic replication is the same as that of the molecular replication of desoxyribosenucleic acid (HERSHEY and CHASE 1953). The specific attachment might be enzymatic (DOUNCE 1953) or some kind of direct physical or chemical force between identical or complementary molecules (WATSON and CRICK 1953). In order to establish replication of the lateral zipper type we must postulate some sort of a specific attachment which occurs sequentially. Thus in figure 4 (d) the next sub-unit added would be in the number 8 position. We must also postulate some effect which causes the two units to separate after the daughter is completed.

With the two assumptions that are used in constructing a model of replication we can explain the production of the heterozygotes without major modifications of the scheme. The phage DNA molecules are extremely long and thin. They are even long compared to the dimensions of the bacterium (LEVINTHAL and FISHER 1953) so that if any of the specific attachment remains in the completed structures we would expect to find many of them lying with homologous units in apposition. If replication starts with the paired structures as parents, we would expect it to start on opposite ends of the two in about half the cases. When the two partially completed daughters meet there will be a specific attachment between them. If we postulate that this cross attachment stops the process, then the structure formed has all of the characteristics of the heterozygotes.

If one accepts the hypothesis that DNA is the genetic material of phage then the structure of this molecule proposed by WATSON and CRICK (1953a) immediately suggests a specific model for the chromosomes of the heterozygotes. There are two intertwined complementary chains in this proposed DNA structure. Every adenine in one chain is attached to a thymine molecule in the other by a pair of hydrogen bands; and every guanine will similarly be attached to a cytosine (in the T2 virus a 5-methyl cytosine) in the complementary chain. In any structure of this type the genetic information must be contained in the sequence of the nucleotides, and in the WATSON and CRICK structure this information is contained twice, since the order of the nucleotides in one uniquely determines the order in the other. This suggests that the DNA

of the heterozygotes have a structure which can be represented schematically by figure 1 b II. The two lines represent the two chains of one molecule, and the solid and dotted portions refer to the parental particles which determine the order of the nucleotides. In the overlap region the complementarity breaks down, but since phage particles are generally heterozygous for only one or two markers the lack of complementarity would probably not be significant for the molecular structure.

This type of model is fundamentally different from normal diploidy since the two chains will not necessarily reassort randomly. We consider (WATSON and CRICK 1953b) a model of replication in which the two chains first separate at one end, and then each of the chains acts as a template for the formation of its complement. The new partial structure would be stable and might tend to break the next bonds of the parental structure. This process would then continue in a zipper-like fashion until two molecules were completed. If this mechanism does apply to the heterozygotes we would have segregation on the first replication and the heterozygotes would not reproduce themselves.

The problem of the formation of the heterozygotes still remains and even if we consider the WATSON and CRICK mechanism of replication the discussion given above concerning *de novo* information seems to apply. There does not seem to be any way in which breakage of already formed molecules could produce heterozygotes of the required structure without introducing very artificial assumptions. The lateral attachment of partially formed complements, although complicated by the helical nature of the structure, seems to present a reasonable possibility.

Several additional predictions follow from the type of model used here. Recombinants are not produced reciprocally by the heterozygotes. The $A^+ B$ recombinants are statistically, but not individually, related to the $A B^+$ recombinants. In single burst experiments HERSHEY and ROTMAN (1949) showed that there is no correlation between the numbers of the reciprocal recombinants from single bacteria although they are equal in the mass culture. VISCONTI and DELBRUCK (1953) explained this lack of correlation by the randomizing effect of growth and extraction from the vegetative pool following mating, but without an excessively large pool it is not clear that these randomizing influences would be sufficient. Another prediction, which has not as yet been testable, is that no chromosomes are broken down during mating. In particular, the chromosomes of the input particles must reappear unchanged in the progeny.

It was shown in the interpretation of the increase of recombinants in lysis-inhibited cultures (LEVINTHAL and VISCONTI 1953) that if we assume a connection between the production of recombinants (i.e., mating) and replication, we can then calculate the size of the vegetative pool from the total burst size. This connection seems to be more strongly indicated by the nature of the heterozygotes; and the number of rounds of mating, m, becomes the number of generations in the vegetative pool. From the rate of increase in recombinants we estimated the rate of growth to be about one generation every two minutes.

This time should be approximately equal to the washout time for the P^{32} in the DNA pool if the vegetative phage are DNA molecules. HERSHEY's (1953a) measurements of this quantity are in approximate agreement with the estimate from the genetic data.

ACKNOWLEDGMENTS

I wish to thank DRS. J. LEDERBERG, S. E. LURIA and N. VISCONTI for the very intimate part they had in the conception of these experiments, and MISS JOYCE MOLIN for her invaluable assistance in performing them.

SUMMARY

In three-factor crosses with T2 phage it was found that particles selected for heterozygosis of the middle marker were generally recombinants for the end markers. This suggests a model for the heterozygotes that involves a small overlapping piece which can be considered as a partial diploid section of a haploid chromosome. Knowing that the heterozygotes lead to recombinants, it is possible to calculate the number of recombinants which would be contributed by this mechanism alone. The calculated value is in agreement with the observed number and it is concluded that heterozygotes are the intermediates in the production of recombinants in phage. In a discussion of the implications of these results it is suggested that the simplest explanation is one in which the heterozygotes are formed *de novo* by the cooperative production of a daughter by two parental structures.

LITERATURE CITED

ADAMS, M. H., 1950 Methods of study of bacterial viruses. Methods of Medical Research. **2**: 1–73. The Year Book Publishers, Chicago.

BELLING, JR., 1933 Crossing over and gene rearrangement in flowering plants. Genetics **18**: 388–413.

DOERMANN, A. H., 1952 The intracellular growth of bacteriophages. I. Liberation of intracellular bacteriophage T4 by premature lysis with another phage or with cyanide. J. Gen. Physiol. **35**: 645–656.

1953 The vegetative state in the life cycle of bacteriophage. Cold Spring Harbor Symp. Quant. Biol. **18**: (in press)

DOUNCE, A. L., 1953 Duplicating mechanism for peptide chains and nucleic acid synthesis. Enzymologia **15**: 257.

HERSHEY, A. D., 1953a The nucleic acid economy of the phage infected cell. Cold Spring Harbor Symp. Quant. Biol. **18**: (in press)

1953b Inheritance in bacteriophage. Advances in Genetics **5**: 89–106. Academic Press, New York.

HERSHEY, A. D., and M. CHASE, 1951 Genetic recombination and heterozygosis in bacteriophage. Cold Spring Harbor Symp. Quant. Biol. **16**: 471–479.

1952 Independent function of viral protein and nucleic acid in growth of bacteriophage. J. Gen. Physiol. **36**: 39–56.

HERSHEY, A. D., and R. ROTMAN, 1948 Linkage among genes controlling inhibition of lysis in a bacterial virus. Proc. Nat. Acad. Sci. **34**: 89–96.

1949 Genetic recombination between host range and plaque type mutants of bacteriophage in single cells. Genetics **34**: 44–71.

LEVINTHAL, C., and H. W. FISHER, 1953 Unpublished results.

LEVINTHAL, C., and M. VISCONTI, 1953 Growth and recombination in bacterial viruses. Genetics **38:** 500–511.

VISCONTI, M., and M. DELBRÜCK, 1953 The mechanism of genetic recombination in phage. Genetics **38:** 5–33.

WATSON, J. D., and F. H. C. CRICK, 1953a A structure for desoxyribose nucleic acid. Nature **171:** 737.

1953b Genetical implications of the structure of desoxyribonucleic acid. Nature **171:** 964.

THE INHERITANCE OF SUSCEPTIBILITY TO THE TERATOGENIC ACTION OF CORTISONE IN MICE [1]

HAROLD KALTER

Department of Genetics, McGill University, Montreal, Canada

Received July 10, 1953

It has been found (FRASER and FAINSTAT 1951) that pregnant mice given cortisone during gestation will give birth to offspring a certain proportion of which have cleft palates. The frequency with which this deformity appears was observed to depend on: 1) the genotype of the treated animal, strain A being "susceptible," C57BL relatively "resistant," to this effect of cortisone; 2) the dose of the hormone administered, too small an amount being ineffective in producing cleft palates, too great an amount causing resorption of the entire litter; and 3) the time during the gestation period when the animal was treated.

The investigation reported here has provided further observations on the genetics of susceptibility to the teratogenic effects of cortisone.

MATERIALS AND METHODS

The animals used in this study were the A/Jax and C57BL/6Jax inbred strains of mice, F_1 females of reciprocal crosses between these strains, and females of the first backcross to strain A. Three or four females and one or two males were kept in each breeding cage. They were fed water and Purina Fox Chow *ad libitum*, supplemented twice a week by lettuce and white bread soaked in milk.

The breeding cages were examined periodically for females which were palpably pregnant and for females with vaginal plugs. All females which were palpably pregnant or had vaginal plugs were placed in separate cages where they were treated and remained for the duration of their pregnancies. After delivery the females were returned to their original breeding cages. The offspring were examined for gross abnormalities and then discarded or fixed in Bouin's solution for future histological study.

Pregnant females were given 2.5 mg of cortisone acetate (11-dehydro-17-hydroxycorticosterone-21-acetate, Merck) for four successive days (called 4×2.5 mg cortisone) intramuscularly in the flank. It had previously been established that this dose of the hormone caused a relatively high incidence of cleft palate with a relatively low frequency of resorption of litters. The fluid in which the hormone is suspended has no gross effect on development (FRASER and FAINSTAT 1951).

[1] This paper is taken from a thesis submitted to the Faculty of Graduate Studies and Research, McGill University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

TABLE 1

Length of gestation in days measured from observation of vaginal plugs to parturition for females of strains A, C57BL (= B), and the F_1 and backcross (BC) generations.

Cross	Length of gestation in days									Total	Mean ± S.E.
	15	16	17	18	19	20	21	22	23		
A × A					5	6	1			12	19.67 ± 0.17
B × B	1			2	14	17	7	3	3	47	19.98 ± 0.19
A × B		1		2	7	5				15	19.00 ± 0.27
B × A		1		2	13	7	6	2		31	19.65 ± 0.22
F_1 × A		1	1	15	14	4				35	18.54 ± 0.15
BC × A				4	6	1				11	18.73 ± 0.18

None of 202 C57BL and 232 strain A offspring of untreated mothers was observed to have a cleft palate without cleft lip. The incidence of spontaneous cleft palate with cleft lip in the offspring of A mice in this laboratory is about five percent. Since this defect is quite different anatomically from that induced by cortisone, and since the treatment does not appear to increase the incidence of cleft palate with cleft lip, offspring affected in this fashion have been excluded from the data presented below.

Observations made in this laboratory have determined that the palatine processes fuse in the normal embryogeny of strain A/Jax early on the fifteenth day of gestation (WALKER unpublished).

RESULTS

The length of the gestation period. For a proper evaluation of the importance of the stage during gestation when the agent is administered, it is necessary that an accurate estimate of the age of the fetus at the time of treatment be known. This age can be calculated in a rough manner by counting back from the time of parturition. In order to do this, however, one must know the length of the gestation period. The commencement of the gestation period can be identified by means of the vaginal plug which, since it is the coagulated secretion of the seminal vesicles of the male (GRUNEBERG 1952), is an infallible sign of copulation. Observations of gestation length in females of various genotypes are presented in table 1. The combined data for the pure strains and for the hybrids are found in table 2. A test of significance shows that the mean gestation lengths for these two groups of animals are quite different ($t = 5.33$, d.f. = 149, $P << 0.001$). Since females were examined each morning

TABLE 2

Length of gestation in days measured from observation of vaginal plugs to parturition for pure strain and hybrid females (data from table 1).

Females	Length of gestation in days									Total	Mean ± S.E.
	15	16	17	18	19	20	21	22	23		
Pure strain	1	2		6	39	35	14	5	3	105	19.70 ± 0.13
Hybrid		1	1	19	20	5				46	18.59 ± 0.12

for several months for the presence of vaginal plugs, it is certain that they were formed not longer than twenty-four hours prior to the time of observation. Since mice tend to ovulate during the early morning (RUNNER and LADMAN 1950) and also tend to copulate then (SNELL *et al.* 1940) the majority of vaginal plugs seen were but six to ten hours old. This means that the values for the length of the gestation period in table 1 were probably slight underestimations. Taking this probable discrepancy into consideration, the mean gestation lengths of the treated animals have, therefore, been reckoned as 20 days for the pure strain animals and as 19 days for the F_1 and backcross (BC) animals.

GRUNEBERG (1952) has emphasized that, because of the great variability of the gestation period and the fairly constant degree of maturity at birth, there must be differences in the rate of development which make the chronological age of an embryo an unreliable indicator of its developmental age. In view of this variability and because the vaginal plug was not observed in some treated females, a precise estimation of the developmental stage treated was not possible for this material. To introduce some degree of uniformity it was decided to estimate the ages of fetuses from pure strain and hybrid females at the time of treatment by counting back from 20 and 19 days, respectively, for all litters.

The unusual occurrence of gestation lengths of as short as 15 and as long as 22 and 23 days have been recorded in table 1. In the case of the shortest period, two live offspring were born, both normal in outward appearance. It is possible that an observational oversight was the cause of imputing so short a gestation length to this pregnancy. Omitting it from the calculation would, of course, raise the figure for the mean gestation length toward 20 days and would not, therefore, invalidate the assigned mean. Concerning the longer pregnancies, normal variation cannot be excluded as their cause.

GENETIC ANALYSIS

Strain differences. The results of administering 4×2.5 mg cortisone to pregnant A and C57BL females confirmed the original impression that these strains respond quite differently to this treatment (table 3). Treatments begun on the eleventh day of gestation produced the highest incidence of cleft palate in both strains. The χ^2 values for days 11, 12 and 13 reveal the differences between these strains in response to the teratogenic effects of the hormone to be real. (All χ^2 tests have been corrected by YATES' method, where appropriate.)

Reciprocal crosses between strains. As the first step in a genetic analysis of this situation, the A and C57BL strains were crossed to each other reciprocally. (Following the usual convention, in all crosses to be mentioned, the female parent is written first.) The results obtained (table 4) show that when strain A (susceptible) animals were used as the female parent ($A \times B$) the frequencies of cleft palate were higher than when the C57BL (resistant) animals were the female parent ($B \times A$). The former cross gave incidences

TABLE 3

Incidence of cleft palate (C.P.) in progeny of pregnant A and C57BL (= B) ♀♀ treated with 4 × 2.5 mg cortisone, begun on various days of gestation.[1]

Gestation day	A × A				B × B				χ^2	P
	Number ♀♀ treated[2]	Number born[3]	Number with C.P.	Percent C.P.	Number ♀♀ treated[2]	Number born[3]	Number with C.P.	Percent C.P.		
8	1	1	0	....	....	....	....	....		
9	....	....	....	....	8	18	3	16.67		
10	1	2	1	....	13	59	8	13.56		
11	10	36	36	100.00	22	75	14	18.67	61.72	$\ll 0.001$
12	4	14	11	78.57	15	69	5	7.25	30.46	$\ll 0.001$
13	4	19	9	47.37	22	100	5	5.00	20.57	$\ll 0.001$
14	8	38	0	0	13	46	1	2.17		
15	5	18	10	55.56	6	25	0	0		
16	1	3	0	....	5	25	0	0		
17[4]	....	....	....	....	6	29	2	6.90		
18[5]	1	6	0	....	6	32	0	0		
19[6]	....	....	....	....	5	21	0	0		
Total	35	137	67	....	121	499	38	....		

[1]This table includes results presented in FRASER and FAINSTAT (1951).
[2]Excluding 10 A and 26 B ♀♀ whose litters were resorbed, aborted, or eaten before examination.
[3]Excluding 4 A × A and 18 B × B offspring whose palates were unexaminable due to cannibalization by mothers.
[4]Treatments begun on this day consisted of 3 × 2.5 mg cortisone.
[5]Treatments begun on this day consisted of 2 × 2.5 mg cortisone.
[6]Treatments begun on this day consisted of 1 × 2.5 mg cortisone.

TABLE 4

Incidence of cleft palate (C.P.) in progeny of crosses A × B and B × A; treatments 4 × 2.5 mg cortisone, begun on various days of gestation (B = C57BL).

Gestation day	A × B				B × A				χ^2	P
	Number ♀♀ treated[1]	Number born[2]	Number with C.P.	Percent C.P.	Number ♀♀ treated[1]	Number born[2]	Number with C.P.	Percent C.P.		
8	....	....	....	....	3	5	1	....		
9	....	....	....	....	7	15	3	20.00		
10	2	12	4	33.33	9	39	0	0	....	0.002[3]
11	11	46	20	43.48	12	82	3	3.66	28.30	≪0.001
12	14	49	9	18.37	14	75	3	4.00	5.44	0.02
13	4	7	1	....	14	93	0	0	....	0.07[3]
14	5	35	0	0	8	47	0	0		
15	2	6	2	....	6	34	0	0		
16	2	9	0	....	2	3	0	....		
17[4]	2	8	0	....	3	23	0	0		
18[5]	....	....	....	....	4	27	0	0		
Total	42	172	36	....	82	443	10	....		

[1]Excluding 9 A and 28 B ♀♀ whose litters were resorbed, aborted, or eaten before examination.
[2]Excluding 27 A × B and 27 B × A offspring whose palates were unexaminable due to cannibalization by mothers.
[3]Probability derived by use of FISHER's exact treatment of the 2 × 2 table (FISHER 1950).
[4]Treatments begun on this day consisted of 3 × 2.5 mg cortisone.
[5]Treatments begun on this day consisted of 2 × 2.5 mg cortisone.

TABLE 5

Incidence of cleft palate (C.P.) in progeny of crosses AB × A and BA × A; treatments 4 × 2.5 mg cortisone, begun on various days of gestation.

Gestation day	AB × A				BA × A				χ^2	P
	Number ♀♀ treated[1]	Number born[2]	Number with C.P.	Percent C.P.	Number ♀♀ treated[1]	Number born[2]	Number with C.P.	Percent C.P.		
6	2	4	0	....	....	....	....	....		
7	2	5	0	....	....	....	....	....		
8	4	20	0	0	4	13	2	15.38	....	0.15[3]
9	5	40	4	10.00	9	24	6	25.00	1.41	0.24
10	13	83	20	24.10	11	44	10	22.73	0.002	0.93
11	15	116	26	22.41	11	71	18	25.35	0.08	0.78
12	15	86	10	11.63	22	158	28	17.72	1.16	0.28
13	5	24	0	0	6	43	1	2.33	....	0.64[3]
14	2	17	0	0	4	36	0	0		
15	4	37	0	0	1	11	0	0		
16	2	15	0	0	2	14	1	7.14		
17[4]	2	20	0	0	2	21	0	0		
18[5]	1	12	0	0	....	....	....	....		
Total	72	479	60	....	72	435	66	....		

[1]Excluding 12 AB and 13 BA ♀♀ whose litters were resorbed, aborted, or eaten before examination.
[2]Excluding 42 AB × A and 30 BA × A offspring whose palates were unexaminable due to cannibalization by mothers.
[3]Probability derived by use of FISHER's exact treatment of the 2 × 2 table (FISHER 1950).
[4]Treatments begun on this day consisted of 3 × 2.5 mg cortisone.
[5]Treatments begun on this day consisted of 2 × 2.5 mg cortisone.

of the defect intermediate between those for the A × A and B × B crosses. The incidences from the B × A cross, however, were lower than those for the resistant (C57BL) strain mice (for day 11, $\chi^2 = 7.71$, $P = 0.005$).

A difference involving a maternal influence, in a situation such as this, may be due to several causes: it may result from inherent differences in the maternal and/or fetal response to the agent, transmitted through the cytoplasm; it may stem from innate differences in response of the mothers, transmitted through the nucleus; it may be due to short-acting cytoplasmically inherited differences in the fetal response. On *a priori* grounds the last is an unlikely explanation for the phenomenon.

First backcross. To distinguish between the former possibilities, F_1 females from reciprocal crosses of A and C57BL animals (AB ♀ ♀, from the cross A × B; BA ♀ ♀, from the cross B × A) were mated to A males. These crosses are designated as AB × A and BA × A, respectively. Table 5 reveals that both types of F_1 females had similar frequencies of affected offspring, a result which would not be expected on the basis of a hereditary cytoplasmic influence. It does indicate that genetic factors in the mother, inherited through the nucleus, are responsible for the maternal effect.

Second backcross. The female offspring of the cross $F_1 \times A$ were mated to strain A males. (This cross is denoted BC × A.) The data pertaining to the frequency of cleft palate in this cross are contained in table 6, which shows an incidence of 56 percent for treatments begun on day 11. The significance of these data will be discussed below.

TABLE 6

Incidence of cleft palate (C.P.) in progeny of cross BC × A; treatments 4 × 2.5 mg cortisone begun on various days of gestation (BC = backcross).

Gestation day	BC × A			
	Number ♀♀ treated[1]	Number born[2]	Number with C.P.	Percent C.P.
8	5	17	10	58.82
9	10	52	17	32.69
10	21	128	71	55.47
11	14	91	51	56.04
12	13	71	15	21.13
13	4	25	0	0
14	2	4	0	....
15	5	41	0	0
16[3]	1	7	0	....
17[4]	7	42	0	0
18[5]	3	15	0	0
Total	85	493	164	....

[1]Excluding 16 ♀♀ whose litters were resorbed, aborted, or eaten before examination.

[2]Excluding 24 offspring whose palates were unexaminable due to cannibalization by mothers.

[3]Treatments begun on this day consisted of 3 × 2.5 mg cortisone.

[4]Treatments begun on this day consisted of 2 × 2.5 mg cortisone.

[5]Treatments begun on this day consisted of 1 × 2.5 mg cortisone.

Pattern of inheritance. The investigation of the mode of inheritance of the character susceptibility to the teratogenic effects of cortisone and that of the character relative fertility (which subject will be discussed later) was complicated by the fact that one cannot usually collect enough data from any one female to classify her for either of these characteristics with any degree of confidence. This fact precluded the application of rigorous mathematical techniques for the analysis of the number of genes involved. However, the data that were obtained have been studied to gain certain indications concerning the situation.

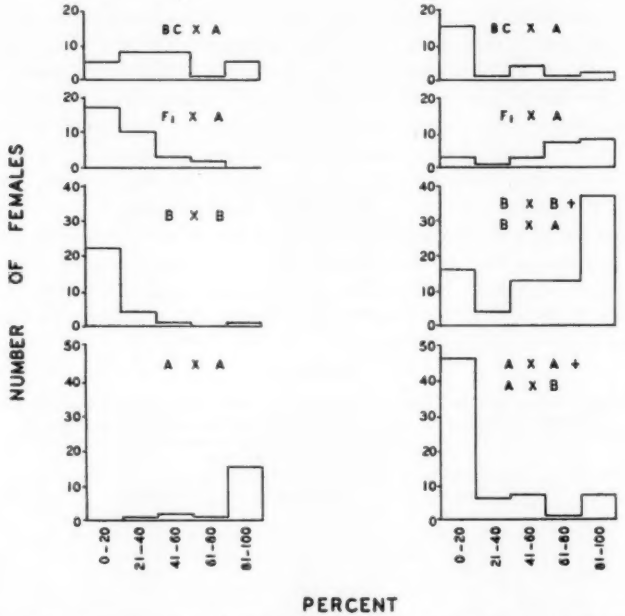

FIGURE 1.—Percentage of total progeny of females of different genotypes with cleft palate (left) and percentage of vaginal plugs which were followed by conception (right) in females of different genotypes.

Histograms constructed from the data concerning the performances of individual females of different genotypes regarding incidence of cleft palate per litter and the frequency with which copulations were followed by an established pregnancy are found in figure 1. The variability of the incidence of cleft palate in the offspring of the backcross females which is illustrated in figure 1 (top left) appears greater than that for females of other genotypes. This is indicative of a segregation of the genetic factors determining susceptibility to the teratogenic effects of cortisone. This increase in variability is consonant with a polyfactorial mode of inheritance of the character. On the

TABLE 7

Frequency of occurrence of pregnancy following isolation with vaginal plugs (V.P.) of females in different crosses (B = C57BL, BC = backcross).

Cross	Number ♀♀ isolated with V.P.	Number pregnant	Percent conceived
1 A × A	65	13	20.00
2 B × B	99	58	58.59
3 A × B	83	17	20.48
4 B × A	65	41	63.08
5 F_1 × A	56	37	66.07
6 BC × A	49	13	26.53

other hand, the portion of figure 1 illustrating the character relative sterility (right half) shows no such difference in degree of variability. The distribution for the backcross females is very similar to that for the strain A females.

RELATIVE STERILITY

One index of the fertility of female mice is the proportion of copulations (i.e., vaginal plugs) that result in palpable pregnancy. This proportion has been shown (FAINSTAT 1951) to be low for A and higher for C57BL females. The results of this investigation confirmed this finding and also indicated that such was the case regardless of the males to which these females were mated (tables 7 and 8). The females employed were roughly equivalent as to age; however, since the strain A females were much less fertile than the C57BL females, the latter became palpably pregnant, and were, therefore, treated, more often than were the A animals.

The fertility of the F_1 females (66%) was not significantly different from that of the C57BL females (59%), which suggests that the genetic basis for fertility may be dominant to that for sterility (table 7, lines 2 and 5; table 8, line 6). The backcross females, however, did not differ significantly from the A animals (26% and 20%; table 7, lines 1 and 6; table 8, line 7).

These values of fertility are in no way to be construed as characterizing any but cortisone-treated females, and may, indeed, be considered the outcome of such treatment, since work proceeding in our laboratory (WALKER personal

TABLE 8

Comparisons of fertility of females of various crosses (B = C57BL, BC = backcross).

Crosses compared	χ^2	P
1 A × A : B × B	17.48	$\ll$0.001
2 A × B : B × A	25.87	$\ll$0.001
3 A × A : A × B	0.007	0.96
4 B × B : B × A	0.22	0.64
5 F_1 × A : A × A	24.60	$\ll$0.001
6 F_1 × A : B × B	0.52	0.47
7 BC × A : A × A	0.34	0.56
8 BC × A : B × B	12.26	<0.001

communication) indicates that entirely untreated strain A females are much more fertile than the treated strain A females, the data for which appear in tables 7 and 8. Untreated C57BL females, however, are approximately as fertile as their treated strainmates (TRASLER personal communication).

CLEFT PALATE AND SEX

No difference between the sexes in proportion of affected animals was detected in the offspring of the cross $F_1 \times A$, the cross from which the largest number of offspring was obtained (table 9).

TABLE 9

Sex distribution of normal and affected offspring of cross $F_1 \times A$ (C.P. = cleft palate).

	Normal	C.P.	Total
♀♀	379	58	437
♂♂	357	54	411
Total	736	112	848

$\chi^2 = 0.002$ $P = 0.93$

DISCUSSION

The inheritance of the character "susceptibility to the teratogenic effects of cortisone" has been shown to be independent of any transmissible cytoplasmic influence and to be wholly transmitted through the nuclear genotype (at least in so far as the differences between strains A and C57BL are concerned). The expression of the character, however, is dependent on an interaction between the genotypes of the mother and fetus. The F_1 hybrids of the cross $A \times B$ had a much lower incidence of cleft palates than did strain A embryos in the same uterine environment. That some portion of the increased resistance was due to hybrid vigor was shown in the cross of resistant strain females to susceptible strain males ($B \times A$), where the incidence of the abnormality in the offspring was significantly lower than that for the cross of resistant strain females to resistant strain males ($B \times B$).

It would appear from figure 1 that a segregation of the genetic factors responsible for susceptibility to the teratogenic effects of cortisone has appeared in the backcross females, as evidenced by the comparative flatness (i.e., increased variability) of the distribution in the histogram representing the cross $BC \times A$. As has been stated above, however, the paucity of results from individual females precluded any attempt to analyze the data statistically. All that can be said, therefore, is that the patterns illustrated in figure 1 (left half) are consistent with the types of distribution found in the transmission of a polyfactorial, quantitative character.

It has been pointed out (FAINSTAT 1951) that there is an apparent inverse relationship in the A and C57BL strains between the two characters susceptibility and fertility. The data for the pure strains and F_1 hybrids are consistent with this interpretation. The backcross animals, however, did not show such

an inverse relationship. They showed a distribution indicative of the occurrence of segregation for the character susceptibility but no such segregation for sterility. This suggests that the two characters are not causally related. The evidence points to the fact that there is no difference in the degree of fertility of treated and untreated C57BL females. On the contrary, however, the difference in this phenomenon between treated and untreated strain A females is marked, their fertility being decreased by more than half by the treatments. This situation may be the result of failure of conception or of preimplantational or early post-implantational destruction of the embryos. The latter possibility would seem to be the more likely since it would be consistent with the greater susceptibility of strain A females to cortisone effects, some of which may be the production of a systemic debility. This interpretation falls down, however, in the consideration of the backcross females, whose offspring had a significantly lower incidence of cleft palates than strain A offspring had (for day 11, $\chi^2 = 21.30$, $P \ll 0.001$), but which were not statistically different in fertility from the A females (table 8). One way out of this dilemma would be to assume that the backcross and A females are susceptible in different degrees to different effects of cortisone. If this were so it would definitely indicate the causal nonrelatedness of the characters.

SUMMARY

Administration of cortisone to pregnant female mice induced the appearance of cleft palates in the offspring. The incidence of the defect was high in strain A (susceptible) animals and comparatively low in C57BL (resistant) animals. Reciprocal crosses between these strains gave incidences of the defect intermediate between those of the pure strains when the female parent was of the susceptible strain, and incidences significantly lower than those for the resistant strain when the female parent was of the resistant strain.

Similar incidences of the defect resulted from matings of the reciprocal F_1 females to susceptible strain males. Thus the factors controlling susceptibility are not cytoplasmically inherited and involve both the maternal and fetal genotypes.

Strain A females were significantly more sterile than C57BL females when mated to males of either their own or the other strain. The F_1 females were as fertile as the C57BL females; the backcross females were as sterile as the A females. The data suggest that susceptibility to the teratogenic effects of cortisone is not causally related to relative sterility.

ACKNOWLEDGMENTS

This investigation was supported by the KATE E. TAYLOR FUND of the BANTING RESEARCH INSTITUTE, Toronto. Cortisone was generously supplied by DR. J. H. LAURIE, MERCK CO., LTD., Montreal. I wish to thank DR. F. C. FRASER, Genetics Department, MCGILL UNIVERSITY, for his reading and criticism of this manuscript and for his sympathetic and stimulating guidance.

LITERATURE CITED

FAINSTAT, T. D., 1951 Hereditary differences in ability to conceive following coitus in mice. Science **114:** 524.

FISHER, R. A., 1950 Statistical methods for research workers. Hafner Publ. Co.: N. Y.

FRASER, F. C., and T. D. FAINSTAT, 1951 Production of congenital defects in the offspring of pregnant mice treated with cortisone. Pediatr. **8:** 527–533.

GRUNEBERG, H., 1952 The genetics of the mouse. Martinus Nijhoff: The Hague.

RUNNER, M. N., and A. J. LADMAN, 1950 The time of ovulation and its diurnal regulation in the post-parturitional mouse. Anat. Rec. **108:** 343–362.

SNELL, G. D., E. FEKETE, K. P. HUMMEL, and L. W. LAW, 1940 The relations of mating, ovulation and the estrous smear in the house mouse to time of day. Anat. Rec. **76:** 39–54.

AN ANALYSIS OF CROSSING OVER WITHIN A HETEROZYGOUS INVERSION IN DROSOPHILA MELANOGASTER [1]

E. NOVITSKI AND G. BRAVER
Department of Zoology, University of Missouri, Columbia, Missouri

Received July 21, 1953

THE study of crossing over between chromosomes differing by an inversion provides information on the capability of chromosomes to synapse and undergo exchange despite the mechanical obstacle imposed by the inversion loop configuration. Extensive information is available in *Drosophila melanogaster* on the frequencies of crossing over outside the inverted region of inversion heterozygotes, but measurement of crossing over within the inversion presents difficulties, since single crossovers are ordinarily lost by the formation of dicentrics or duplication-deficiency chromosomes. Frequencies of double crossing over in very long inversion heterozygotes are known; this type of heterozygote, however, constitutes the least interesting and informative case because the chromosomes may pair in reverse direction without the formation of a typical inversion loop. The ideal conditions for this sort of study, then, include the use of a medium-sized inversion centrally located in a chromosome arm with an appreciable pairing segment on both sides of the inversion and some specially devised constitution such that crossovers both within and outside of the inversion yield genetically detectable products. These conditions have been fulfilled by the use of a tandem metacentric compound X chromosome heterozygous for the *dl-49* inversion. An analysis of crossing over in this compound is presented below.

CONSTRUCTION OF THE TANDEM METACENTRIC COMPOUND

The compound X chromosome used in these experiments was made up according to a method described previously (NOVITSKI 1954) and the appropriate scheme is given in figure 1 of that work. Bar males were mated to females heterozygous for a chromosome in normal sequence, except that it carried the *dl-49* inversion, and a second X chromosome which was in inverted order, with the base of *In(1)EN* and the tip of *In(1)sc*^{4}, and which had, in addition, a short arm consisting of the proximal segment of the X chromosome derived from the B^{S} translocation. Crossing over between the base of the first chromosome and the short arm of the second should give rise to a metacentric compound X chromosome with its components tandemly arranged, and an egg with such a chromosome, if fertilized by a Y-bearing sperm, would produce a non-Bar female, an exceptional type. From a large

[1] This investigation was supported by a research grant C-1578C from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

number of progeny examined from the above mating, five such exceptions were found. Their subsequent genetic behavior indicated them to be of the desired type.

The *dl-49* chromosome carried the mutants, *y*, *v*, *f* and *car*; the other carried *y* and *m*. The relative positions of these mutants, the extent and location of the *dl-49* inversion, and the configuration achieved by this compound after complete synapsis is given in figure 1 under type 1. The proportions of the various regions are drawn approximately to scale from the genetic data on the X

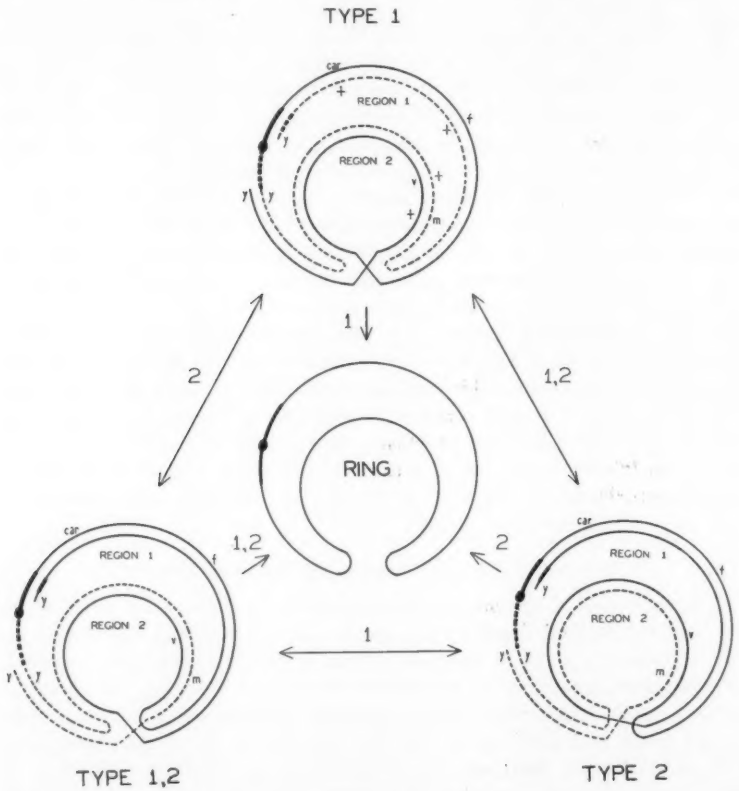

FIGURE 1.—The pairing configurations of the three types of compounds, assuming complete pairing. Type 1 is the original, types 1,2 and 2 are the derived types. Region 1 refers to all the chromosome except the *dl-49* inversion, which is region 2. The numbers by the arrows indicate the position of the exchange that will make the indicated transformations. The positions of the mutants and their normal alleles are given for type 1, as it was synthesized, but only the loci are indicated for the other two types since the exact position of the mutants will depend on the position of the exchange responsible for the transformation. The heterochromatic regions are represented by thickened lines.

chromosome found in BRIDGES and BREHME (1944). For convenience, the regions adjacent to the centromere and outside of the *dl-49* inversion are referred to jointly as region 1 and the inverted region as region 2.

CROSSING OVER IN THE TANDEM METACENTRIC, TYPE 1

Region 1. As illustrated in figure 1 under type 1, a single crossover in region 1 between the two arms of the tandem metacentric may produce a ring chromosome consisting of a single X chromosome. It should be noted that in this compound there are two distinct kinds of single exchanges, one in which the two chromatids involved are attached to the same centromere and another in which the two chromatids are attached to sister centromeres. A schematic diagram of the latter requires the four-strand stage; all crossover products from it are inviable. The two-strand schematic, as in figure 1, is adequate to show the consequences of the first type of exchange which produces viable

TABLE 1

Types of progeny produced from matings of females with the tandem metacentric compound X chromosome heterozygous for dl-49 to B, X-Y males.

1. Noncrossovers:		
a. Matroclinous	*y* ♀♀	2456
b. Patroclinous	*B* ♂♂	2804
2. Single crossovers:		
a. Region unidentifiable	*B* ♀♀	322
b. Exchange between		
y* and *dl-49	*y v f car* ♂♂	3
dl-49* and *f	*y m f car* ♂♂	90
f* and *car	*y m car* ♂♂	132
***car* and centromere**	*y m* ♂♂	165
3. Double crossovers:	*y v* ♀♀	2
	y m ♀♀	1
	y v f car ♀♀	2

products. For simplicity, the term exchange will usually be used here only with reference to the kind giving viable products; however, estimates of exchange frequencies must take both kinds into consideration.

Exchanges occurring in the various sections of region 1 may be readily identified. Table 1 gives the frequencies of the various types recovered from a mating of females with the type 1 chromosome to X-Y, *B* males. Single crossovers are recovered as single rings in both sexes, whereas the compound may be recovered only in the female sex; consequently the relative frequency of single rings may be calculated as $(322+390)/2(2456+5)+(322+390) = 712/5634 = 12.6\%$.

Of the 390 single rings recovered as males, only 3 resulted from exchange between *y* and the distal breakpoint of *dl-49*. All the others resulted from exchange between the proximal break and the centromere. The relative proportions of exchange in the regions *y* to distal breakpoint *dl-49*, proximal breakpoint *dl-49* to *f*, *f* to *car*, and *car* to the centromere are, respectively, 0.8%, 23.1%, 33.8% and 42.3%.

Region 2. A crossover within the *dl-49* inversion, region 2, does not produce an immediately identifiable product. Instead, a new chromosome type, that drawn in figure 1 as type 1,2 results. It can be seen upon inspection of the type 1,2 configuration that it will not produce single ring chromosomes by a single exchange; a double exchange, one in region 1 and one in region 2, is required. It is labelled type 1,2 for this reason.

It was anticipated that a female with type 1,2 would produce fewer single-ring-bearing progeny than those with type 1. Accordingly, progeny tests were set up to determine the relative frequencies of types 1 and 1,2 chromosomes from mothers with type 1. Since there was no specific expectation for the frequency of production of single rings from type 1,2, progeny of females showing a lower than average production of rings were tested. It was found that only in cases where a female had produced very few (not more than two) single-ring-bearing progeny was this characteristic of low ring production repeated by her progeny. In every case females suspected of carrying the type 1,2

TABLE 2

The percentages of cultures of females carrying types 1 and 1,2 chromosomes with 0, 1,2 and 3 or more single rings produced by crossing over and the average number of female progeny per culture for those cases where complete counts were necessary.

No. of rings per culture	Chromosome type 1		Chromosome type 1,2	
	%	Average yield	%	Average yield
None	0.6	29.3	73.9	48.4
One	1.5	44.6	21.7	59.3
Two	2.4	48.7	4.3	63.0
Three or more	95.5	....	00.0	....

chromosome were checked by tests of their progeny. In a few cases the tests were not completed; these were classified as doubtful.

Of 1313 progeny of type 1 females tested, 116 were retested because they produced three or less single-ring-bearing F_1 and 46 proved to carry the type 1,2 chromosome. In two cases the tests were inconclusive and a third case involved a still different chromosome type, 2, to be described below, resulting from a double exchange in the type 1 compound. That the criterion for the classification of the type 1,2 chromosome is reliable is shown by table 2. It should be noted that the cultures of type 1 females producing no rings tend to be somewhat lower in productivity than average.

The 46 instances of conversion of type 1 compound into a type 1,2 out of 1313 tests give a frequency of recovery of crossovers within the *dl-49* inversion in this type configuration of 3.5%. The two doubtful cases would increase this figure to 3.7% if they were, in fact, crossovers also.

It has been possible, by further analysis of the crossover types 1,2, to determine where in the *dl-49* inversion the exchange took place. Discussion of these results is deferred until the characteristics of all new crossover types have been presented.

CROSSING OVER IN THE TYPE 1,2 COMPOUND

Region 1. An exchange in region 1 of the type 1,2 produces a new compound, type 2, so named because it will produce single ring chromosomes by single exchange in region 2 (fig. 1). It is difficult to distinguish between types 1,2 and 2 because they behave in much the same way. It is not possible for this reason to present any specific estimate for the conversion from type 1,2 into 2; i.e., the crossover frequency in region 1 of the type 1,2 chromosome. It is known however that this conversion does take place. In tests of 540 progeny of females carrying the 1,2 chromosome, five were shown definitely to carry the type 2 chromosome. The nature of this identification is described below.

Region 2. An exchange in this region results in the conversion of type 1,2 back to type 1. Since these two types can be distinguished without undue difficulty, a more or less precise figure can be arrived at for the frequency of conversion. Of the 540 tests of type 1,2, there were 12 cases of conversion to type 1, and two additional cases where the classification was doubtful, giving a frequency of 2.2 to 2.6%.

Regions 1 and 2. Crossovers in regions 1 and 2 simultaneously will produce a single ring. Although these doubles are rare, an estimate may easily be made of their frequency since the single rings are recovered as individuals and progeny tests are unnecessary. The tests of the 46 females that proved to carry the type 1,2 compound yielded 14 progeny with single rings to 2363 with the compound. The latter figure must be multiplied by two to correct for the loss of half of the compounds by fertilization by an X-bearing sperm; the frequency of production of single rings is therefore $14/(4726 + 14)$, or 0.3%.

CROSSING OVER IN THE TYPE 2 COMPOUND

Region 1. A crossover in region 1 converts type 2 into type 1,2 (fig. 1). No estimate has been made of the frequency of this change.

Region 2. A single in region 2 gives rise to a single ring. Crossing over in this region (*dl-49*) is not common; the identification of a chromosome as type 2 rests upon the distribution of the mutants *f* and *car* in the recovered rings. Reference to figure 1 will show that the specific allele of *f* and *car* present on the single ring is determined by the location of the exchange in region 1 responsible for the initial occurrence of the type 1,2 compound. Consequently, all single rings from a given female will be identical with respect to these mutants. Thus, a given type 2 compound will produce rings all of which carry *car*, or all with *f car*, or all with neither, unlike types 1 and 1,2 which produce rings with all three possible combinations in a single test. For example, a set of thirty daughters from one female with a type 2 compound produced the following kinds of single rings: 29 *m car*, 4 *v m car* and 3 *v car*. The alleles within *dl-49* will, of course, vary within a culture since they will depend upon the position of the exchange giving rise to the ring. This will be discussed in more detail in the section on the distribution of exchanges within the *dl-49* inversion. Nine females, subsequent tests of whose progeny showed that they carried the type 2 compound, produced 667 compound X progeny to 21 rings,

or 1.5%. Tests in later generations gave a lower value, presumably because of the presence of a number of type 1,2 chromosomes newly formed by crossing over in region 1 of the type 2.

Regions 1 and 2. No reversions to type 1 by a double in type 2 were observed in the small number of tests of type 2 compounds.

THE DISTRIBUTION OF EXCHANGES IN REGION 2

The position of the crossover in the *dl-49* inversion responsible for the conversion of type 1 into type 1,2 cannot be determined immediately. The genetic

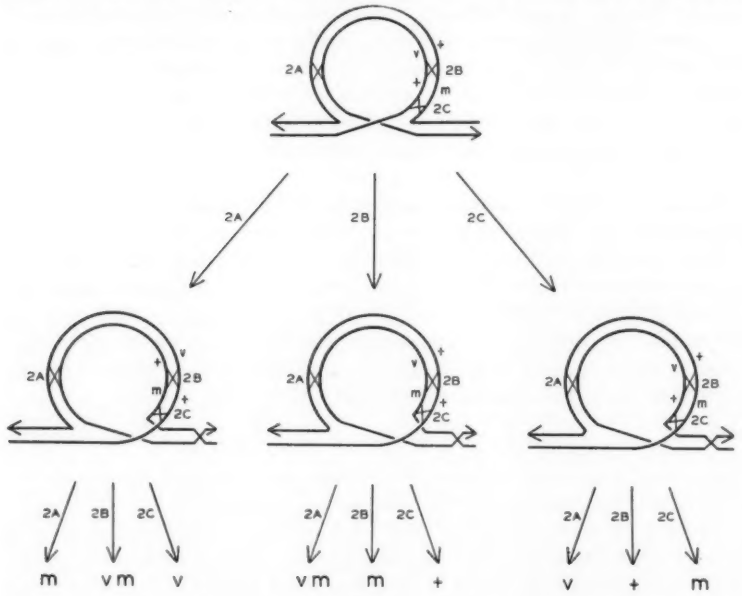

FIGURE 2.—The method for locating the position of exchanges within the *dl-49* inversion, showing how different initial exchanges alter the distribution of mutants carried by the single rings produced in later generations.

composition of the kinds of rings produced by type 1,2, however, do indicate the location of the original region 2 exchange. This is diagrammed in figure 2. Region 2 is divided into three subregions: from the breakpoint of *dl-49* to *v*, from *v* to *m*, and from *m* to the other breakpoint. These are labelled 2A, 2B, and 2C, respectively. The chromatids ending in arrows are those joined to the centromere. From the figure it is seen that an exchange in each of the three regions gives rise to three possibilities with respect to the distribution of the mutants and their normal alleles on the two chromatids, although all three are structurally type 1,2. In each of these types, once again exchange may occur in each of the three subregions. In combination with an exchange out-

side of the inversion, single rings may be produced. The mutants carried by the rings will be different for the different combinations of exchanges, but more diagnostic, perhaps, is the absence of one of the possible combinations in each of the three sets. Thus, if the original exchange was in region 2A, none of the single rings appearing from later crossing over should be non-*m* non-*v*. On the other hand, if it was in 2B, rings with *v* only should be absent and if in 2C, the *v m* class should be absent.

The exchange in region 1 necessary for ring production in addition to the one in region 2 may occur simultaneously with the latter as a double, or it may occur in an intervening generation, converting the type 1,2 into type 2. In either case the analysis is the same, although the latter is more efficient since rings are produced more readily by type 2 than by type 1,2.

In practice, the above expectations have been fulfilled and it has been possible to specify the location of the original exchange in 15 crossovers in the *dl-49* inversion. Ten gave a distribution of single rings indicating that the original exchange must have been in 2A, one proved to be a 2B exchange and four were of the 2C type. An additional one gave only two crossovers, both of which were *m*, suggesting that here too the original exchange was located in 2A, since this is the most frequent class from the 2A group and least frequent from the other two.

TABLE 3

The distribution of exchanges in the type 1,2 configuration when one occurs in region 1 and the second in region 2. The numbers in parentheses give expected distribution if the exchanges were occurring singly.

Region 1	Region 2			Total	Expected
	2A	2B	2C		
breakpoint-*f*	6	2	1	9	(6.7)
f-car	6	2	1	9	(9.9)
car-centromere	11	0	0	11	(12.4)
Total	23	4	2	29	(29.0)
Expected	(22.5)	(3.2)	(3.3)	(29)	

When the type 1,2 is converted into type 2, single rings are produced with a higher frequency, as noted above. From these type 2 compounds it is possible to designate the specific region of exchange directly involved in the production of the rings, provided only that it is possible to determine the exact constitution of the type 2 chromosome. Of 510 rings from type 2 females, 395 came from crossing over in 2A, 57 from crossing over in 2B and 58 from crossing over in 2C.

A small amount of data has been collected on the location of the exchanges occurring simultaneously in the two regions 1 and 2 when rings are produced from the type 1,2 compound. These cases are given in table 3, along with the expected distribution calculated from the relative frequencies of recovered crossovers in region 1 in type 1 and in region 2 in type 2.

DISCUSSION

The use of this specially constituted chromosome which is not only a compound X chromosome but has effectively two inversions, one very long involving an entire chromosome arm and the other *dl-49*, may be open to question because its synaptic properties could be basically different from those of two unattached homologues differing only by the *dl-49* inversion. To some extent there must be differences; it is nevertheless surprising that the similarities in exchange values between this type and others from which information can be obtained are so striking.

Crossing over outside of the *dl-49* inversion in normal chromosomes has been studied extensively (STURTEVANT and BEADLE 1936). Crossovers between y and the left break of *dl-49* are rare (0.5%), as they are in the compound. The other regions agree in their crossover rates also, but the comparisons depend upon the conversion of observed rates into exchange frequencies. As pointed out earlier, half of all exchanges give rise to inviable zygotes. In tetrads of the type 1 compound, when a ring is formed by exchange in region 1 there is an unaffected type 1 compound sister chromatid not shown in figure 1 which has been drawn for simplicity as the two-strand stage. The estimation of the exchange frequency must take all these into account; calculated most simply the exchange frequency would be four times the number of single rings recovered, since only one quarter of all exchanges in region 1 produce single rings, divided by a total which has been corrected for the loss of zygotes due to inviable exchange products and to fertilization of compound X eggs by X-bearing sperm. Arithmetically this becomes $4 \times \text{rings}/(2 \times \text{compound X's} + 3 \times \text{rings})$. If we accept the best value (table 1) for the frequency of production of rings as $2 \times 390 = 780$ (since it has been shown by NOVITSKI (1954) that the recovery of ring-bearing females from crosses of this sort tends to be depressed and that the best estimate of ring production is made by doubling the ring-bearing male class) and the frequency of compounds as 2461, then the exchange frequency in region 1 becomes 0.43 which would correspond to a crossover value of 0.215 in an ordinary *dl-49* inversion heterozygote. More complicated calculations which take into account other products, such as the double exchanges, give the same value for the exchange frequency. This is much higher than the 0.13–0.15 value found in such heterozygotes (STURTEVANT and BEADLE 1936). While it is conceivable that exchange is more frequent in the compound than in unattached or ordinary attached-X chromosomes heterozygous for *dl-49*, the high exchange value is probably best interpreted as a manifestation of nonrandom disjunction, and it should be noted that this particular phenomenon, an excessive recovery of rings from the tandem metacentric compound, has been well established (NOVITSKI 1951). If the excess of rings is about the same as in previous experiments, then the calculated exchange value of 0.43 may be about 50% higher than the true value, which would be 0.29, a figure in close agreement with the exchange values from the more orthodox chromosome types.

It is of particular interest that the high rate of exchange between the in-

version and the centromere and the low rate distal to the inversion, found in simple heterozygotes and in attached-X heterozygotes, is found also for this compound where the terms proximal and distal pairing lose their significance since the proximal region of each arm pairs with the distal region of the other. It can be concluded that the relative frequency of crossing over in each of these regions is primarily a function of the nature of the region and that the modification of crossover values by the position of the region with respect to the centromere (the "centromere effect") is of secondary importance.

The frequency of exchange within *dl-49*, region 2, in the experiments reported here is complicated by the fact that the identification of a compound as a crossover, type 1,2 from type 1, depends on progeny tests. Some fraction of the eggs laid in the same generation as those carrying the crossover compounds must have carried rings, or inviable products, and so would not have been tested; these, nevertheless, must be added to the total of 1313 tested to give a more valid estimate of the total sample. The determination of this increment involves drawing the type 1 compound in the four-strand stage, and calculating, from the observed numbers of the different types produced, and from the frequency of ring production from the type 1 compound (15.8%), the number of rings and inviable zygotes. The adjusted total is about 2050, which gives a frequency of exchange of from 9.2 to 9.6%, depending on whether the two doubtful cases are included. If, in these calculations, nonrandomness is assumed to be operating, the values are increased slightly, to 9.6 and 10.0%. STURTEVANT and BEADLE (1936) made an estimate of the exchange frequency in *dl-49* using an attached-X heterozygous for the inversion. In this configuration, crossovers within *dl-49* produce inviable zygotes and the additional depression of the attached-X progeny below the patroclinous male class compared to a control series without *dl-49* was taken as a measure of the exchange frequency. Their experiments gave a value of 12%.

Before considering the rates found in the other two types, it should be pointed out that the three types are not synaptically equivalent. A close examination of types 1,2 and 2 in figures 1 and 2 will reveal that, assuming complete synapsis, the basal region of each arm pairs with the distal region of the same arm, forcing the *dl-49* regions in each arm into a loop. If it is hypothesized that the depression of crossing over within an inversion results from an inability of the chromosomes to synapse simultaneously outside of the inversion and also within the inversion because the relatively inverted segments must become reoriented with respect to each other for pairing, this difficulty in the formation of an inversion loop would disappear in types 1,2 and 2 since here complete pairing outside of the inversion would facilitate loop formation in each arm and synapsis within the *dl-49* region could be completed simply by the juxtaposition of the loops in each arm. It will be shown below that the data do not support this idea; it is presented simply to emphasize the caution that must be observed in equating the three types to each other.

The best estimate for the frequency of exchange in the inversion, region 2, in the type 1 compound is 9.6 to 10.0%. In a way identical to that described

above for the type 1 compound, exchange frequencies in region 2 may be obtained for the other two types. The calculated exchange value in the type 1,2 compound ranges from 7% to 9%, depending on whether two doubtful cases are included, and the exchange value for this region in the type 2 compound is 5%. In both of the latter cases exchange would have been facilitated, giving an expectation above 10% if the mechanism for the supression of crossing over were that described in the preceding paragraph. The differences in the exchange values between type 1 and the other two types are not unequivocal, being based on low numbers of crossovers derived from crosses made at different times. Nevertheless the 5% figure for the type 2 compound strongly suggests that there is in fact a depression since this is based on the recovery of single rings which, as has been shown above, are very likely being recovered preferentially because of the nonrandom effect. It is possible that pairing in region 2 is less often achieved in the types 1,2 and 2 than in type 1, despite the presence of the true inversion configuration in the latter and not in the former.

Examination of the distribution of exchanges within the *dl-49* inversion does not lead to the conclusion that there are extensive synaptic difficulties because of inversion loop formation. Of sixteen crossovers analyzed, four were found to have occurred between the miniature locus and the adjacent breakpoint. Furthermore, of the 510 crossovers in type 2 giving rise to rings, eleven percent occurred in this region. This high frequency in the region adjacent to the breakpoint may be related to the low frequency from *y* to the inversion; i.e., a low degree of synapsis in the latter region may allow for greater synapsis near the breakpoint within the inversion. The relative frequencies of exchange in the three sections of region 2 in the type 2 compound may be compared with expectation in the following way. The approximate genetic lengths of regions 2A, 2B and 2C on a normal chromosome are 22, 4 and 5 units respectively. The relative lengths are 0.71, 0.13 and 0.16. The frequencies of exchange in those regions are 395, 57 and 58, or, as decimals, 0.77, 0.11 and 0.11. An agreement between the two sets of figures is reached by assuming simply a slight deficiency of exchange in region 2C and a slight excess in region 2A.

It might be argued that this random distribution holds because pairing within the *dl-49* region is complete when it occurs (except, of course, for the sections immediately adjacent to the breakpoint) and that the reduction in crossing over comes from competition between the inverted and uninverted segments for pairing partners. This would give rise to interference between simultaneous pairing in the two regions and could be detected as a reduced number of doubles below expectation. For the type 1 compound the data are meager. If the frequency of exchange in region 1 is taken as 0.43 and that in region 2 as 0.10, the frequency of doubles should be 0.043. Examination of all possibilities of double exchange in the type 1 configuration shows that only one out of every sixteen gives the type 2 compound. Of the 1313 tests of progeny of type 1 compound bearing mothers, only one type 2 compound was

identified when five might have been expected. There are two reasons why fewer than the expected number would have been observed. In the first place, the difficulty in making a simple distinction between types 2 and 1,2 may have led to the inclusion of a few of the former with the latter. Secondly, it has been mentioned earlier that the exchange value in region 1 of type 1 is probably in error by as much as 50% of the true value, since it is based on rings derived from the type 1, a class subject to nonrandom disjunction. If this value is corrected, the expectation becomes three rather than five.

Additional information comes from the type 1,2 compound. The frequency of exchange in region 2 is about 0.08 and that for region 1 can again be taken as 0.43. The expected frequency of double exchanges is simply the product of the two, or 0.034. Taking into consideration all possible combinations of double exchanges occurring in the four-strand stage, one-eighth of the total doubles, or 0.004 of all tetrads, should give rise to rings. The observed frequency was 0.003, which may be corrected to about 0.002 by including in the total a figure for the frequency of inviable zygote production by single exchanges based on the frequencies given above. Note that in this case no correction need be made for the nonrandom effect because both calculated and observed values would have to be altered in the same proportion. This comparison indicates that if there is any interference between exchanges in regions 1 and 2 in the type 1,2 compound, it is probably no more than that which would be expected if there were no inversion present.

Further evidence for the absence of extensive competition between the *dl-49* region and the rest of the chromosome in the type 1,2 compound is given by the breakdown of the doubles into the specific subregions involved (table 3). Although only 29 cases were analyzed, there appears to be a distribution of exchanges in region 2 similar to the distribution when no exchange occurs in region 1, and vice versa. Also, there is no apparent tendency for an exchange in a specific part of region 2 to occur preferentially with an exchange at a specific point in region 1.

The essential points that must be considered in an attempt to develop a self-consistent scheme to explain the behavior of this inversion heterozygote are: (1) a reduction of exchange value in the *dl-49* inversion to about one quarter that found in the same region when no inversion is present, similar in magnitude in both the attached-X and tandem metacentric compounds which pair in quite different ways, (2) a high level of exchange between the proximal break of *dl-49* and the centromere, irrespective of whether the inversion is heterozygous in normal chromosomes, attached-X or tandem metacentric compounds and, contrarily, a low value between *y* and the distal breakpoint in all three cases, (3) a distribution of the exchanges that occur within the *dl-49* region not much different from that expected from a comparison with the standard map, except for a small depression near the breakpoints of the inversion and (4) the apparent absence of any strong interference between exchanges occurring within and outside of the *dl-49* region. In addition, there is an indication of a lower value of exchange in the *dl-49* region found in the

two types with intra-arm pairing compared to the value from the type 1 compound which is a true inversion heterozygote.

All of these relationships can be rationalized if it is simply assumed that the synaptic tendency along the euchromatic length of the chromosome is very weak and ordinarily becomes manifest after homologous regions have been brought into proximity by stronger (but not necessarily specific) pairing centers located in the heterochromatic regions adjacent to the centromeres. These pairing regions would be, presumably, similar or identical to those postulated by COOPER (1951) on cytological grounds. According to this idea, the distal heterochromatin of the sc^4 inversion at the tip of one arm of the tandem metacentric, by pairing with the heterochromatin region near the centromere, is responsible for bringing the normally basal regions together, whereupon the euchromatic pairing tendency takes over and causes a nearly normal degree of synapsis, and crossing over, in that region. The homologous *dl-49* regions will be brought closer together by this and in some cases, presumably in about 25% of the total, are sufficiently near to allow those euchromatic regions to synapse. The effect of the inversion, then, in reducing crossing over is not so much a consequence of the formation of the inversion loop as it is of the interruption of the continuity of euchromatic pairing. The shorter euchromatic segment from *y* to the distal break pairs relatively infrequently. Any reduction of crossing over in region 2 of the types 1,2 and 2 compounds would be explainable on the basis of the intra-arm pairing; here the arm with the heterochromatic tip derived from sc^4 pairs with its own base and the *dl-49* regions of the two arms synapse only when they happen by chance to be suitably disposed to do so.

While this scheme is adequate to account for the results in these experiments, it is clear that it cannot be extended to include cases of inversion heterozygotes studied in maize (RHOADES and DEMPSEY 1953), where it has been quite clearly demonstrated that the Drosophila type of crossover suppression, particularly in the regions adjacent to the inversion, is absent. It could be surmised that there may be basic differences in the constitution of the chromosomes in the two forms, possibly with respect to the presence of knobs in maize. In any case, the postulate of primary heterochromatic pairing centers functional in normal oogenesis of Drosophila is one which is subject to direct experimental test.

SUMMARY

Crossing over has been studied in a specially constituted chromosome, a tandem metacentric compound X chromosome, in *Drosophila melanogaster*, in which it is possible to measure rates simultaneously within a heterozygous inversion, *dl-49*, and outside the limits of the inversion. The frequency of exchange within the inversion is reduced to about twenty-five percent of the amount expected for the same region without an inversion. Crossovers within the inversion appear to be randomly distributed, except for a slight depression near the breakpoints of the inversion, and there is no striking interference

between crossing over outside of the inversion and within it, with respect to either the overall frequencies or the position of the crossovers. This compound is unstable and by crossing over gives rise to new types with different synaptic properties, whose crossover rates have also been studied. It is suggested that the similarities and dissimilarities in crossover values between the tandem metacentric, its derivatives, ordinary attached X chromosomes, and simple chromosomes, all heterozygous for the *dl-49* inversion, are understandable if it is assumed that the pairing of chromosomes is initiated by the heterochromatic regions, with a weaker euchromatic pairing tendency coming into effect after homologous euchromatic regions find themselves in proximity.

ACKNOWLEDGMENTS

The writers wish to express their thanks to MRS. NORMA BRAVER and MISS EVELYN BARBOUR whose patience and industry added appreciably to the number of tests carried out in this work, and to DR. D. L. LINDSLEY and PROF. M. M. RHOADES whose criticisms have helped immeasurably in the preparation of the manuscript.

LITERATURE CITED

BRIDGES, C. B., and K. S. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Inst. of Wash., Publ. 552. 256 pp.

COOPER, K. W., 1951 Studies on spermatogenesis in *Drosophila*. Yearb. Amer. Phil. Soc. for 1951: 146–147.

NOVITSKI, E., 1951 Non-random disjunction in *Drosophila*. Genetics **36:** 267–280.

1954 The compound X chromosomes in Drosophila. Genetics **39:** 127–140.

RHOADES, M. M., and E. DEMPSEY, 1953 Cytogenetic studies of deficient-duplicate chromosomes derived from inversion heterozygotes in maize. Amer. J. Bot. **40:** 405–423.

STURTEVANT, A. H., and G. W. BEADLE, 1936 The relations of inversions in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21:** 554–604.

CHROMOSOME NUMBERS IN SOMATIC TISSUES OF MOUSE AND MAN

B. E. WALKER AND E. R. BOOTHROYD
Department of Genetics, McGill University, Montreal, Que., Canada

Received July 22, 1953

IT has long been accepted as a valid generalization, subject to certain recognized exceptions, that the number of chromosomes is the same in all somatic cells of an organism. The most commonly recognized types of exceptions are polyploidy and polyteny, which have been found to occur in the cells of differentiated tissues in a variety of animal and plant species. Since these types of variation in chromosome number involve whole genomes, however, they represent balanced systems and are not difficult to fit into the general theories on the genetic control of development.

Direct evidence on chromosome numbers in differentiated tissue is often not available, because of the lack of mitoses, but some indirect evidence supports the contention that the number of chromosomes is constant. BOIVIN, VENDRELY and VENDRELY (1948) believe that the desoxyribose nucleic acid (DNA) is constant in the cells of a species, a view supported by MIRSKY and RIS (1949). SWIFT (1950), studying the amount of Feulgen-positive material in individual nuclei of various tissues of the mouse, found the quantity of DNA to be constant or to be some multiple of the basic amount. This was taken to mean that the amount of chromatin was constant from cell to cell except for cases of polyploidy.

Within the last few years the question of the constancy of the chromosome number has once again come under review (cf. HUSKINS 1947) with reports of somatic reduction in plants (HUSKINS 1948; HUSKINS and CHENG 1950; HUSKINS and CHOUINARD 1950; WILSON, HAWTHORNE and TSOU 1951) and of aneuploidy in mammals (MELANDER 1950; TIMONEN 1950; TIMONEN and THERMAN 1950; THERMAN and TIMONEN 1951) which are difficult to reconcile with classical theory. MELANDER's case of aneuploidy in a "triploid" rabbit might be passed over as being due to the unusual nature of the material, but TIMONEN's (1950) report of a high frequency of aneuploidy in man cannot be so considered.

TIMONEN (1950) counted 1000 chromosome groups in Feulgen squash preparations of human endometrium, 100 from each of ten individuals, and found a range of chromosome numbers from 4 to more than 100. The distribution was found to be bimodal, with "the highest peak lying at 20–25 chromosomes and a much lower one at 45–50 chromosomes" (THERMAN and TIMONEN 1951). Measurements of 2000 nuclei gave a distribution of volumes which was similar to the distribution of chromosome numbers. Estimations based on the size of the metaphase plates in various human embryonic tissues, as well as some actual counts, likewise showed "that most of the cells were hypoploid." These findings have led THERMAN and TIMONEN to suggest that a

high frequency of aneuploidy, with most cells hypoploid, may be common in higher animals. The fact that most studies of chromosome numbers in higher animals have been made on germinal tissue is advanced to explain why such a high frequency of aneuploidy has been overlooked in the past. They suggest that gene action in these forms may take place largely through the various glands, so that the lack of some of the chromosomes in cells of differentiated tissues may be relatively unimportant.

Because of the broad theoretical implications of THERMAN and TIMONEN's findings, should they be confirmed and also found to hold true for other warm-blooded animals, it was felt that a similar study should be made on another mammal. Consequently, the present investigation was undertaken, using the mouse, *Mus musculus*, as the experimental material. Human endometrium was also used for direct comparison with THERMAN and TIMONEN's results. Preliminary results have been published elsewhere (BOOTHROYD and WALKER 1952).

MATERIALS AND METHODS

All materials in this study were fixed in 95% alcohol–glacial acetic acid (3:1). Semi-permanent preparations of material stained and mounted in aceto-carmine were made by tilting the slide and allowing WILSON's (1945) Venetian turpentine medium to run under the cover glass to displace the stain. The cover glass was then pressed down and its edges sealed with nail polish.

The four methods used for preparing mouse tissue were (1) placing pieces of intestine from day-old mice under a cover glass and spreading them by tapping the cover glass; (2) dissecting off a thin membrane from the outer circumference of the intestine of day-old mice; (3) dissecting off the epithelial layer from the cornea of adult mice (GAY and KAUFMANN 1950); (4) placing pieces of adult mouse intestine in fixative and mechanically stirring the fixative (the "spun tissue method"). The tissues in methods (1) and (4) were stained with aceto-carmine just before the cover glass was added, and in methods (2) and (3) the aceto-carmine was allowed to flow under the cover glass after the membranes were laid on the slide in fixative in an unfolded condition. The mechanical stirring referred to was achieved by spinning the tissue for 4 minutes in 1 cc of fixative with a gear wheel seven millimeters in diameter powered by a "Tiny Atom" motor with two 1½ volt flashlight batteries in series. Human endometrium, obtained during dilatation and curettage for metrorrhagia, was prepared by the "spinning method" and by the Feulgen squash technique.

All mitotic figures of sufficient clarity were drawn, and counts were made from the drawings. No revision of an initial count was made, but some of the figures were drawn separately by both authors to get some indication of the reliability of the counts.

RESULTS

Aceto-carmine squashes of the intestine of day-old mice (method 1) provided an abundance of easily-counted mitoses. Results (table 1 and fig. 1) ap-

TABLE 1

Chromosome counts from mouse and human tissues.

Number of chromosomes per group	Frequency of groups						
	Day-old mouse intestine			Adult mouse tissues			Human endometrium "spun" (4)
	Squash (1)[1]	Membrane (2)		Corneal epithelium (3)	Intestine, "spun" (4)		
		Original	Check[2]		Original	Check[2]	
1	5						
2	1						
3	1						
4	2						
5	3						
6	1						
7	1						
8	2						
9	3						
10	5						
11	3						
12	6						
13	3						
14	2						
15	3						
16	4						
17	4						
18	5						
19	4						
20	2						
21	1						
22	3						
23	3						
24							
25	1						
26							
27	1						
28	2						
29	3						
30	1	1					
31	1						
32		1					
33	3						
34	6	2				1	
35	9	2	1			1	
36	7	6	3	6	1		
37	4	9	2	5	6	1	
38	7	5	2	6	6	12	
39	4	11	6	7	8	6	
40	6	9	1	10	4	7	
41	3	7	3	2	5	4	
42	6	3	1	3	5	1	1
43		2					3
44	2	2	1		3	1	1
45		1		1	1		3
46	1	1			1		2
47							4
48		1					4
49							4
50							5
51							1
52							3
53							3
54							
55							1
56							1

[1]Numbers in brackets refer to methods as listed under Materials and Methods.

[2]Check counts by the junior author to give an estimate of the amount of variation which might be attributed to counting error (not included in graphs).

TABLE 1 (*continued*)

Number of chromosomes per group	Frequency of groups						
	Day-old mouse intestine			Adult mouse tissues			Human endometrium "spun" (4)
	Squash (1)[1]	Membrane (2)		Corneal epithelium (3)	Intestine, "spun" (4)		
		Original	Check[2]		Original	Check[2]	
57							
58							2
59							1
60							
61							
62							1
....							
75			1[3]				
Mean ± S.E.	24.5 ± 1.14	38.9 ± .39	38.8 ± .51	38.8 ± .31	39.7 ± .57	39.0 ± .32	49.5 ± .75
S.D.	13.2	3.1	2.2	2.0	3.6	1.9	4.7

[1]Numbers in brackets refer to methods as listed under Materials and Methods.
[2]Check counts by the junior author to give an estimate of the amount of variation which might be attributed to counting error (not included in graphs).
[3]Probable tetraploid cell; omitted from calculations.

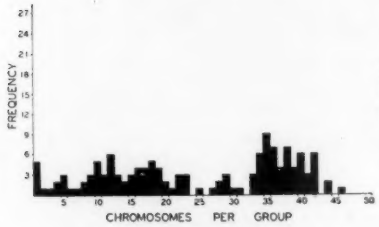

FIGURE 1.—Counts from day-old mouse intestine prepared by the squash method.

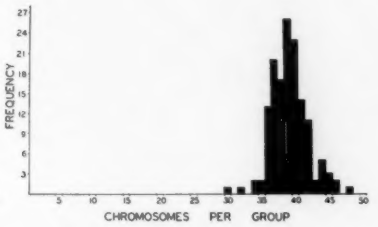

FIGURE 2.—Graph combining figs. 3, 4, and 5.

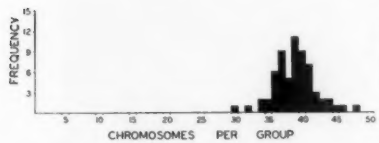

FIGURE 3.—Counts from mounts of day-old mouse intestinal membrane.

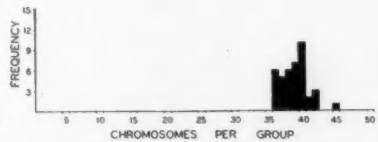

FIGURE 4.—Counts from mounts of adult mouse corneal epithelium.

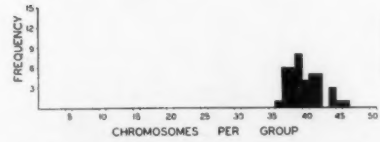

FIGURE 5.—Counts from adult mouse intestine prepared by the spinning method.

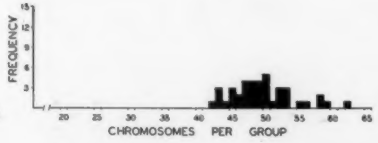

FIGURE 6.—Counts from human endometrium prepared by the spinning method.

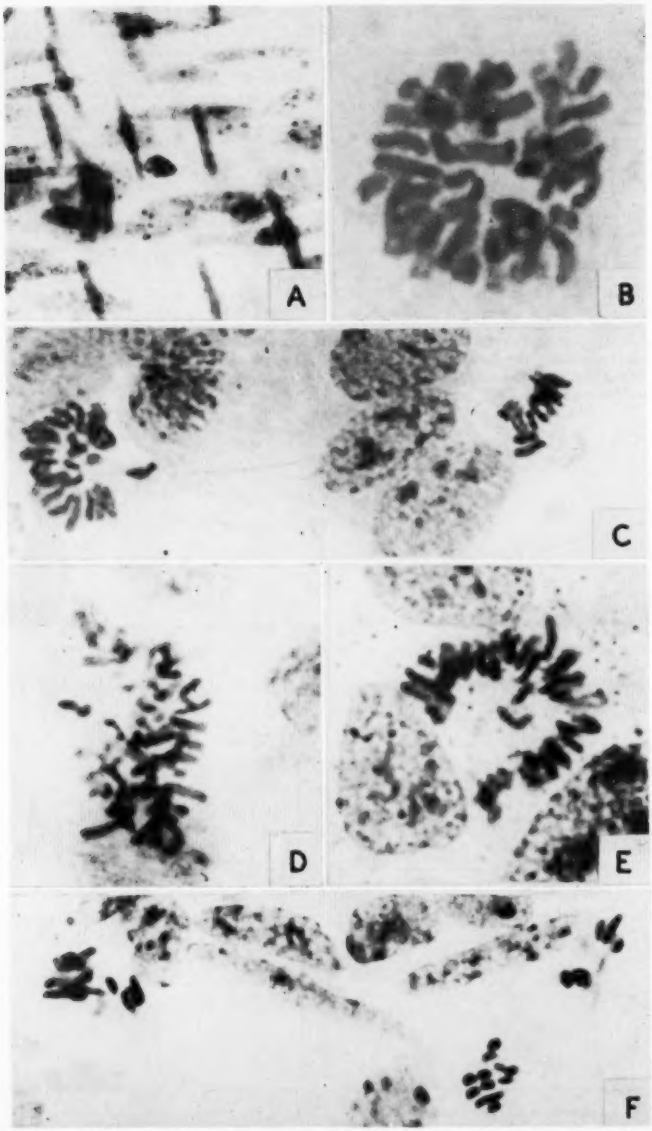

PLATE I

peared to indicate that a condition of aneuploidy existed in somatic tissues of mice similar to that reported for human tissue by THERMAN and TIMONEN.

However, as a precautionary measure, it seemed desirable to use some other method in which the possible factor of chromosome groups breaking apart during treatment could be better controlled. Consequently, flattened membranes were used. In the pieces of membrane taken from newborn mouse intestine (method 2), the cells away from the edges are intact, as can be deduced from the orientation of nuclei in plate I A. Counts of chromosome numbers made independently by the two authors in tissue prepared in this way gave results which differed strikingly from those obtained by the squash method (table 1 and fig. 3). In the membranes no figures were found with less than 30 chromosomes. Extending the study to adult mouse tissue, counts were made on sheets of corneal epithelium (method 3), and the deviation from diploid was found to be even less, with the counts ranging from 36 to 45 chromosomes per group (fig. 4).

The technique of dissecting off membranes cannot be applied to very many tissues, so a more widely applicable method was sought for preparing tissues without causing widespread cell breakage. The procedure of fragmenting tissue by agitation in fluid (method 4) produced remarkably little breakage of chromosome groups (plate I B) if treatment was stopped when small, but macroscopic, bits of tissue were abundant. These bits of tissue tend to flatten on the slide like miniature membranes, although this tendency varies with the type of tissue used. Among the 80 figures counted in "spun" material only three showed evidence of having been broken (plate I C). The counts obtained from spun adult mouse intestine were closely grouped around 40 (table 1 and fig. 5).

Because methods involving membranes and spun material gave different results from those obtained by squashing, it was decided that human endometrium should be investigated using the spinning method. The distribution of counts from endometrium is shown in table 1 and figure 6. There was a complete absence of small chromosome groups, and among the figures clear enough to count, (e.g., plate I D) none fell below 42.

PLATE I

A. Small portion of a mount of day-old mouse intestinal membrane showing the regular arrangement of oblong muscle nuclei. A few rounded mesothelial nuclei can also be seen. ca. 700 ×.

B. A group of approximately 39 chromosomes found in a spun preparation of adult mouse intestine. ca. 2600 ×.

C. Adjacent chromosome groups in adult mouse intestine prepared by the spinning method. The two chromosome groups (approximately 29 and 13 chromosomes) in this field are considered to have originated from one metaphase plate. ca. 1600 ×.

D. A chromosome group from human endometrium prepared by the spinning method. ca. 2000 ×.

E. A broken-ring metaphase plate from a squash preparation of day-old mouse intestine. ca. 2100 ×.

F. Small aggregations of chromosomes in close association in a squash preparation of day-old mouse intestine. ca. 1300 ×.

As a further check on the chromosome numbers in human tissue, endometrium was prepared by the Feulgen squash method as used by TIMONEN, with the difference that less pressure was used, to avoid scattering the chromosomes. This made accurate counts impossible, except in a few cases, but good estimates could usually be made. The minimum number of chromosomes was estimated for each of the first 50 metaphases found, classified simply as more than 40, more than 35, and less than 35 chromosomes. The following results were obtained: more than 40, 44 cells; more than 35, 4 cells; less than 35, 2 cells. Of the two which had less than 35 chromosomes, one cell was obviously broken and in the other the chromosomes were strung out. In twelve cells which were clear enough for counting, the counts ranged from 44 to 48 chromosomes per cell.

DISCUSSION

The squash technique, when used on mammalian somatic tissues, causes rupturing of chromosome groups, the percentage of cells ruptured being dependent in part on the particular squash technique used. The first evidence for dispersion was seen in the squash preparation itself. Table 2 shows counts on all closely associated chromosome groups, first on the assumption that they are true hypoploid groups and then assuming closely associated groups to be a single group which had been broken apart (e.g., plate I F). It is interesting to note how the latter assumption leads in most cases to counts approximating forty. TIMONEN (1950) said that, in their work, all obviously broken groups were bypassed, but they did not offer any criteria for identifying these obviously broken groups. The arrangement of the chromosomes immediately comes to mind as a possible method of determining the completeness of the group. A solid ring as compared to a broken ring in a metaphase plate would be an example. However, late prophases contribute many of the counts and they cannot be identified in this manner, nor can the metaphase itself if it is not oriented in a polar view. It is easy to understand why chromosome arrangement is not widely discussed in relation to making counts, since figures that are well enough spread to provide ease of counting are generally pushed out of shape, or broken open as with a broken-ring metaphase plate (e.g., plate I E). The new methods used in this study give a minimum of figure distortion, but do so at the expense of easy counting. The choice had to be made

TABLE 2

All closely associated chromosome groups from squashes of baby mouse intestine.

Counted as separate groups[1]	9	6	11	5	5	10	19	18	29	22	12
	2	35	12	4	4	15	1	16	13	20	16
	27		16	20	9	11	15	8			
				13	18	3	1				
							10				
Counted as one group	38	41	39	42	36	39	46	42	42	42	28

[1]**The vertical columns represent closely associated groups.**

between unbroken groups, and groups that were well enough spread to provide high accuracy of counting. It was decided that the risk of counting error was more acceptable than the risk of error from breakage. Independent counts on the same cells by both authors show that in most cases the error was small, usually less than three chromosomes.

The main evidence against the reliability of the squashing method comes from a comparison of the squash method with the other three methods used. The most accurate comparison would be between intestinal squash preparations on the one hand and intestinal membrane plus spun intestine on the other hand, since spun intestine included mainly the mucosa which separated more easily than the muscle layers. However, since no significant differences exist between the three new methods (see P values in table 3), they can be considered together. Table 3 shows that the mean from squash counts is very significantly different from the mean of the combined counts from the three other methods. The difference in results is quite apparent without any need to refer to statistical analysis, as a glance at figure 1 and figure 2 will show. Upon finding that 58 counts out of 134 fell below 20 with one method and that none of the 143 counts made with other methods fell below 30, the conclusion was reached

TABLE 3

Significance of the difference between means.

Items compared	d.f.	t	P
Squashed intestine with membranes and spun	275	11.6	<.001
Intestinal membrane with spun intestine	101	1.116	.3
Spun intestine with corneal epithelium	78	1.308	.2
Intestinal membrane with corneal epithelium	101	0.163	.9

that the squash technique is an unsatisfactory method for investigating variations in chromosome numbers. It can safely be concluded that there is no large and frequent deviation in chromosome number in the following mouse tissues: intestinal mesothelium, longitudinal and circular muscle layers of the intestine, intestinal mucosa and corneal epithelium.

Evidence now accumulated indicates that the aneuploidy reported by THERMAN and TIMONEN to occur in human endometrium was an artifact produced by their technique. Although they did not give actual frequencies, it was determined by measuring TIMONEN's (1950) frequency histograms that approximately 791 of his 1,000 counts were below 40 chromosomes (the intermode of their distribution) and 209 above. In the present study no counts of less than 40 were obtained in " spun "tissue. Comparing the results of the present investigation with those of THERMAN and TIMONEN on the basis of frequencies above and below 40 chromosomes, the chi square test gives a probability of $< .001$ ($\chi^2 = 124$, 1 d.f.) that the two samples originate from one population. The most reasonable conclusion, therefore, is that THERMAN and TIMONEN were counting broken chromosome groups. Their interpretation of variations in nuclear volume as evidence of corresponding variations in chromosome number also appears open to question, especially in the light of SCHRADER and

LEUCHTENBERGER's (1950) demonstration that in Arvelius different sized nuclei contain protein proportional to their volumes, but their desoxyribose nucleic acid content and chromosome number remain constant.

Although the high frequency of low hypoploid numbers obtained by the squash technique can be considered a product of the method, even the counts obtained from " spun " tissue and membranes were not uniformly diploid. The problem remains, therefore, of deciding whether the variation found represents nothing more than counting errors, or whether some variation in chromosome number actually exists. That counting errors occurred is certain. Counts made independently by the two authors on 35 of the same cells from " spun " mouse intestine differed, on the average, by 2.3 chromosomes per group. However, some real variation may also occur. HSU (1952), in his study of human cells in tissue culture, found some aneuploidy, although deviations from the diploid were much less frequent and less extreme than those reported by THERMAN and TIMONEN. Of 124 cells from spleen cultures which HSU studied, 91 were diploid, one was hypotetraploid, and the remaining 32 contained 44 to 47 chromosomes. His remarkably fine preparations leave little room for counting error, so that it may safely be stated that about a quarter of the cells deviated from the diploid by one to four chromosomes. HOWARD and SANDER (G. F. SANDER personal communication) found similar minor fluctuations in chromosome number in mouse cells.

Limitations of the squash technique should be recognized by workers using this method, and if the cells they are working on are tightly cemented together, possible fracturing of mitotic figures should be anticipated. For studies where this dispersion could be misleading, methods used here might provide successful alternatives. Membrane mounts give the most controlled results, but it is only occasionally that suitable membranes are available. The spinning method is undoubtedly more widely applicable.

SUMMARY

Chromosome counts performed on somatic tissue in the mouse showed wide deviations from diploid in the direction of hypoploidy when the tissue was prepared by the squash technique, but only slight deviations when the tissue was prepared by certain other methods. It was thus shown that the squash technique had broken apart many of the mitotic figures during the mounting of the tissue.

Hypoploidy from squashed human endometrium, as reported in the literature, was studied by another method, and again a discrepancy was found which indicated that the squash technique tended to break up chromosome groups.

It was concluded that in the mammalian somatic tissues studied by the more controlled methods, most of the deviations from diploid were due to counting error, although some of the minor deviations might have been due to aneuploidy.

ACKNOWLEDGMENTS

The authors wish to thank DR. J. S. HENRY and DR. H. B. BOURNE for providing the human material used in this study.

LITERATURE CITED

BOIVIN, A., R. VENDRELY and C. VENDRELY, 1948 L'Acide désoxyribonucléique du noyau cellulaire, dépositaire des caractéres héréditaires; arguments d'ordre analytique. Compt. rend. Acad. sci. **226:** 1061–1063.

BOOTHROYD, E. R., and B. E. WALKER, 1952 Somatic chromosome numbers in mice. Genetics **37:** 567.

GAY, HELEN, and B. P. KAUFMANN, 1950 The corneal epithelium as a source of mammalian somatic mitoses. Stain Tech. **25:** 209–216.

HSU, T. C., 1952 Mammalian chromosomes in vitro. I. The karyotype of man. J. Hered. **43:** 167–172.

HUSKINS, C. L., 1947 The subdivision of the chromosomes and their multiplication in non-dividing tissues: possible interpretations in terms of gene structure and gene action. Am. Nat. **81:** 401–434.

1948 Segregation and reduction in somatic tissues. I. Initial observations on *Allium cepa*. J. Hered. **39:** 311–325.

HUSKINS, C. L., and K. C. CHENG, 1950 Segregation and reduction in somatic tissues. IV. Reductional groupings induced in *Allium cepa* by low temperature. J. Hered. **41:** 13–18.

HUSKINS, C. L., and L. CHOUINARD, 1950 Somatic reduction: diploid and triploid roots and a diploid shoot from a tetraploid Rhoeo. Genetics **35:** 115.

MELANDER, Y., 1950 Chromosome behavior of a triploid adult rabbit. Hereditas **36:** 335–341.

MIRSKY, A. E., and H. RIS, 1949 Variable and constant components of chromosomes. Nature **163:** 656–667.

SCHRADER, F., and CECILIE LEUCHTENBERGER, 1950 A cytochemical analysis of the functional interrelations of various cell structures in *Arvelius albopunctatus* (De Geer). Exptl. Cell Research **1:** 421–452.

SWIFT, H. H., 1950 The desoxyribose nucleic acid content of animal nuclei. Physiol. Zool. **23:** 169–198.

THERMAN, EEVA, and S. TIMONEN, 1951 Inconstancy of the human somatic chromosome complement. Hereditas **37:** 266–279.

TIMONEN, S., 1950 Mitosis in normal endometrium and genital cancer. Acta obst. et gynec. Scandinav. **31** (Suppl. 2): 1–88.

TIMONEN, S., and EEVA THERMAN, 1950 Variation of the somatic chromosome number in man. Nature **166:** 995.

WILSON, G. B., 1945 The Venetian turpentine mounting medium. Stain Tech. **20:** 133–135.

WILSON, G. B., MARY E. HAWTHORNE and TEMAY TSOU, 1951 Spontaneous and induced variations in mitosis. J. Hered. **42:** 183–189.

FREQUENCY OF CHROMOSOME ABERRATIONS PRODUCED BY FRACTIONAL DOSES OF X-RAYS IN TRADESCANTIA[1]

DALE STEFFENSEN AND T. J. ARNASON

Biology Department, Brookhaven National Laboratory, Upton, L. I., N. Y. and Department of Biology, University of Saskatchewan, Saskatoon, Canada

Received July 25, 1953

THEORIES based on results from fractional dose and time-intensity X-ray experiments using Tradescantia inflorescences have become important cornerstones for interpretations of many irradiation experiments. The major evidence has been amassed and theoretical considerations developed by SAX (1939, 1940 and 1941), FABERGÉ (1940), LEA and CATCHESIDE (1942), CATCHESIDE, LEA and THODAY (1946a and b) and CATCHESIDE (1948). According to these workers most chromosome breaks in irradiated Tradescantia restitute or undergo reunion soon after their production and such breaks do not reopen. Because of this rapid reunion or restitution the frequency of two-hit aberrations (i.e., chromatid interchanges, dicentric chromosomes and centric rings) is higher at high intensity than at low intensity. The experiments of SAX on the effects of fractional X-ray dosage have shown that after about one hour from the time of irradiation breakage-ends no longer participate in reunions.

Recently LANE (1951) has questioned most of these findings and their resulting interpretations and has rejected the theory of rapid reunion. LANE's data on reunions presented in his graph 5 show that the highest frequency of breaks was produced by a single dose of 360 r. Thereafter a progressive decrease in the frequency of aberrations occurred when two 180 r doses were separated by 1, 2 and 4 hours.

Reunion frequency was higher when dose-fractions of 180 r each were separated by 6 or 8 hours than when the interval was 4 hours. When the time interval was 8 hours the frequency of reunions was almost as high as that obtained with a single dose of 360 r. The apparent sharp rise in reunions in the 8-hour fractional material led LANE to reject the theory of rapid reunion of X-ray breaks. In his view chromosome breaks produced in the resting nucleus by X-rays may remain open for several or many hours, restitution and reunion being delayed until the beginning of prophase.

The low reunion frequency in the 4-hour fractionation group is attributed to physiological effects of the first radiation fraction on the chromosomes. The chromosomes respond to the radiation by becoming less liable to further breakage by X-rays administered at this time. This newly developed resistance to breakage is highest after about 4 hours but has almost disappeared by 8 hours.

[1] Most of this work was carried out while both authors were at University of California, Berkeley, California. A part of the work was done at the Brookhaven National Laboratory under auspices of the U. S. Atomic Energy Commission and a part was done at the University of Saskatchewan.

LANE attributes the high reunion frequency observed when dose-fractions are 8 hours apart to persistence of open breaks produced by the first dose fraction until after the second group of breaks has appeared. LANE concluded that the chances of reunion are thus very similar in cells given a single dose of 360 r and of cells given two doses of 180 r separated by an interval of 8 hours.

SAX and LUIPPOLD (1952) have repeated LANE's 1951 experiments with *Tradescantia paludosa*. They used a continuous 360 r dose and three fractionations, (2 doses of 180 r in each) the interval between fractions being 4, 8 and 12 hours. A single dose of 180 r was used to establish a base line. The number of aberrations was higher from single than from divided 360 r doses in these experiments. The frequencies of reunions in the 4, 8 and 12 hour fractionated sets were very similar and were approximately equal to those produced by a single dose of 180 r multiplied by 2. These authors failed, therefore, to confirm LANE's essential findings, namely: (1) very low reunion frequency in the 4-hour fractionated-dose material and (2) high reunion frequency in the 8-hour fractionation group. LANE (1952) raised the following objections when he questioned the experimental procedure of SAX and LUIPPOLD: Failure to control temperature after irradiation; use of different experimental material; different X-ray intensity; and different time period between irradiation and fixation.

Firm establishment of results from fractionated X-ray dose experiments is sufficiently important to radiobiological theory that experiments were performed to repeat LANE's 1951 experiment. In these experiments a particular effort was expended to overcome the objections raised by LANE concerning SAX and LUIPPOLD's procedure. The experiments here presented had three general objectives: (1) to repeat LANE's 1951 experiment as closely as was possible. (2) To include two additional irradiation periods of 12 and 24 hours along with single doses of 180 r and 360 r at all fractionation periods to be certain that nuclear sensitivity was constant. (3) To examine cell samples of adequate size and with repetitions so the data would not be seriously questioned because of possible sampling or experimental errors.

MATERIALS AND METHODS

Material used in the study was *Tradescantia paludosa* (clone 5 of SAX). The plants were potted in soil and only young inflorescences (excised from plant) were used. All buds within each experiment were fixed at the same time in ethanol acetic acid (3:1). Samples, depending on the treatment, were irradiated at zero time (3 days before fixation); and at 4, 8, 12 and 24 hours later. These correspond respectively to a post-irradiation time of 72, 68, 64, 60 and 48 hours. Chromosomes were stained with carmine and examined at the first microspore division from permanent slides. Only clear metaphase figures were scored.

The X-radiation, administered with a General Electric Coolidge tube operated at 100 KVP and 3 milliamps was filtered through 1 mm of aluminum. The distance from the target to the inflorescence was 31 cm in experiments

TABLE 1

The effect of fractional dosage on the frequency of chromosome aberrations.

Dose	Time of irradiation (hours)		Exp. no.	Chromosomes scored	Dicentrics and centric rings		Interstitial deletions		Chromosome breaks	
					Percent	χ^2	Percent	χ^2	Percent	χ^2
"Continuous" 360 r	0		1	5502	5.72	0.11	7.67	6.67	7.42	1.80
	0		2	5826	5.66	0.29	6.73	1.16	6.62	0.82
	0		3	1242	5.80	0.003	6.52	0.60	5.56	3.42
	8		1	3288	5.60	0.32	6.90	0.19	8.70	14.67
	12		2	2760	6.59	2.73	6.96	0.09	6.52	0.69
	24		2	930	5.05	0.97	7.74	0.53	5.81	1.72
	24		3	1464	6.56	1.31	5.94	2.79	5.12	6.96
TOTALS				21,012		5.74		12.02		30.09
Grand means with standard errors.					5.83 ± 0.17		7.10 ± 0.18		6.94 ± 0.41	
P values at 6 D.F.					.50–.30		.10–.05		<.001	
	1st	2nd								
Fractional doses 180 r—180 r	0	4	1	2016	4.71	0.82	4.66	3.70	6.60	4.68
	0	4	2	4806	4.60	1.15	5.35	0.96	5.14	1.46
	0	8	1	3258	4.42	0.12	7.15	12.35	6.05	0.34
	0	8	2	2310	4.42	0.10	5.89	0.21	4.24	7.12
	0	12	1	3936	3.89	1.53	6.63	6.21	7.06	1.62
	0	12	2	4356	4.34	0.02	5.46	0.39	5.72	0.40
	0	24	2	3228	3.81	1.76	4.34	10.30	3.87	16.37
TOTALS				23,910		5.51		34.12		21.99
Grand means with standard errors.					4.30 ± 0.13		5.68 ± 0.34		5.55 ± 0.34	
P at 6 D.F.					.50–.30		<.001		<.001	
Single 180 r	0		3	3084	2.20	0.75	2.89	0.07	2.46	1.51
	4		3	2724	2.39	0.04	3.01	0.41	3.08	0.58
	8		3	4608	2.56	0.23	2.82	0.005	2.69	0.34
	12		3	3188	2.73	1.02	2.70	0.13	2.98	0.32
	24		3	1626	2.15	0.58	2.71	0.69	3.26	1.02
TOTALS				15,230		2.63		1.31		3.79
Grand means with standard errors	(2 × for base line values)				2.45 ± 0.13		2.80 ± 0.14		2.84 ± 0.14	
P at 4 D.F.					.70–.50		.90–.80		.50–.30	

1 and 2, and 30 cm in experiment 3. At these distances the intensity of irradiation as measured with a Victoreen dosimeter was 51 r/minute in experiments 1 and 2, and 59 r/minute in experiment 3. The Victoreen instrument was also used to measure the total irradiation received by the inflorescences. Readings recorded were within the 5% error of the dosimeter and verified the calculated doses which are given in the data.

The " continuous " doses of 360 r as implied by the quotation marks were not actually continuous. In experiments 1 and 3, doses were given in two 180 r fractions separated by one minute. In experiment 2 the doses were separated by 2 minutes. This time interval permitted recording the actual doses received although such a procedure was probably unnecessary because calculated doses were well within the 5% accuracy of Victoreen readings. These short fractionations served to lower slightly the number of breaks available for reunion for only a minute or two during the treatment. Any difference in the frequency of two-hit aberrations between these short fractionations and a continuous dose probably would go undetected with even large sample numbers. For simplicity of presentation these short fractionations are referred to as " continuous " doses of 360 r.

The temperatures during irradiation were $23 \pm 0.4°C$, $22.5 \pm 0.5°C$ and $23.7 \pm 0.7°C$ for experiments 1, 2 and 3 respectively. The 0.7 to 1.2° higher temperatures in experiment 3, which would be expected to yield slightly fewer observed breaks (SAX 1947 and CATCHESIDE 1948), were compensated for by a higher dose intensity of 59 r/minute. The higher intensity should bring up the yield of two-hit aberrations to those of experiments 1 and 2. As a result dicentrics and rings should be homogeneous in all experiments. Probably one-hit aberrations would not be homogeneous and would occur at a lower frequency in experiment 3 than in experiments 1 and 2.

After irradiation, inflorescences were transferred to a chamber. The average daily illumination in experiment 1 was 11.3 hours and 12.2 hours in experiments 2 and 3 which approximated natural light conditions in March and April 1952. Until the material was fixed temperatures were maintained at $23.6 \pm 1.6°C$, $22.2 \pm 2.1°C$ and $22.0 \pm 2.0°C$ in experiments 1, 2 and 3 respectively.

RESULTS

The data from these three groups of treatments are given in table 1. The first group of data is from inflorescences exposed to " continuous " 360 r doses. The second group in table 1 was obtained from inflorescences given two doses of 180 r spaced by 4, 8, 12 and 24 hours. The final group was from material irradiated with single doses of 180 r at 0, 4, 8, 12 and 24 hours. The base line of SAX (1941) was obtained from these single 180 r doses by multiplying the frequency of each type of chromosome aberration by two. The three general categories of chromosome aberrations tabulated in table 1 are: dicentric chromosomes and centric rings (reunions), interstitial deletions (minutes) and chromosome breaks. Chromatid aberrations were so infrequent that they were ignored in the scoring.

When tested by the chi square method for homogeneity within groups, dicentrics and centric rings were homogeneous in each case as indicated by P values (see CATCHESIDE *et al.* (1946a) for method of calculation) in table 1. The vertical lines in figure 1 are confidence limits of means at the 95% level. They were derived from SNEDECOR (1946) in the " values of t table " and are presented for dicentrics and centric rings. The grand means and their confidence limits for centric reunions, chromosome breaks and interstitial deletions are separately recorded in table 2. As indicated by the means and confidence limits of .95 found in figure 1 and table 2, the " continuous " 360 r group was

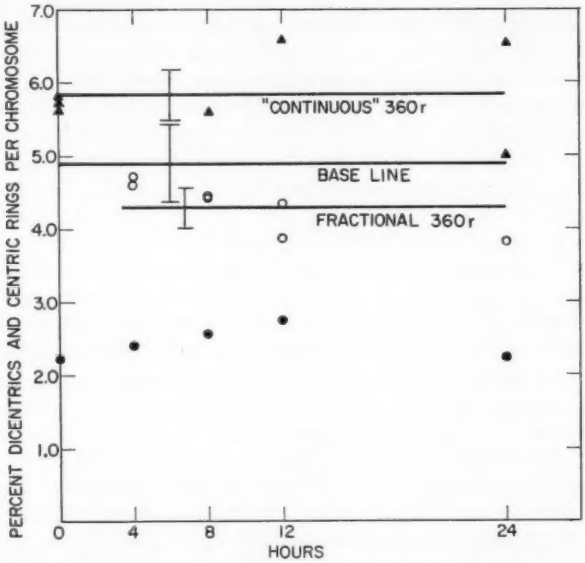

FIGURE 1.—The horizontal lines represent grand means of each treatment group. The base line consists of the grand mean of single 180 r samples multiplied by two. The vertical lines describe confidence limits of each mean at the 95% level. Individual sample points are: ▲, "continuous" 360 r; ○, fractional 360 r (180 r–180 r); and ●, single 180 r.

significantly higher than both fractionated 360 r and base line (2 × single 180 r) groups. The means of fractionated and base line groups did not differ significantly from each other, lying within the 95% limits.

From figure 1 it is apparent that the parameter of the base line estimated from these data could by chance be above or between the various points of the fractionated doses. Because of the overlap of fractionated 360 r and base line means and the homogeneity within both, it is concluded that none of the time interval frequencies were significantly different from the base line. However with increased time intervals between dose-fractions there was a downward trend of the frequency of dicentrics and centric rings (figure 1). Whether

the trend is real or due to chance alone cannot be determined with the chi square test.

Within " continuous " 360 r doses and single 180 r doses interstitial deletions were homogeneous (table 1). Fractionated 360 r doses however had excessive variation. The major chi square contribution came from the two samples, where time between doses was 8 and 24 hours. The second repeat with the 8-hour interval deviated little from the mean which tends to discount any significant increase of interstitial deletions. With the 24-hour split dose however it is not clear whether the sample is significantly lower or if the deviation is due to sampling error.

Grand means of interstitial deletions in " continuous " doses of 360 r are significantly higher ($P < .01$) than those of the fractionated and base line groups.

TABLE 2

The frequency of chromosome aberrations in Tradescantia microspores following "continuous" 360 r, fractional 360 r and single 180 r (× 2) doses of X-rays. Grand means and their confidence limits at the 95% level are expressed in percent of total chromosomes.

	"Continuous" 360 r	Fractional 360 r	Base line (2 × aberrations of single 180 r)
Dicentrics and centric rings	5.83 (5.49–6.17)	4.30 (4.04–4.56)	4.90 (4.38–5.42)
Interstitial deletions	7.10 (6.74–7.46)	5.68 (5.00–6.36)	5.61 (5.05–6.17)
Chromosome breaks	6.94 (6.12–7.76)	5.55 (4.87–6.23)	5.67 (5.11–6.23)

The aberrations scored as chromosome breaks were acentric fragments and acentric rings (× 2). Excluded from chromosome break counts were acentric fragments which came from chromosomes observed as dicentrics and centric rings. The assumption was made that each pair of fragments from these reunion chromosomes always united to form single acentric fragments.

A within-group test for homogeneity in table 1 showed that " continuous " and fractionated 360 r doses were not homogeneous for chromosome breaks, while the single 180 r group was homogeneous. This excess variation may have resulted from sampling errors or more likely scoring errors. Some variation could be due to the fact that samples were irradiated at different temperatures. The samples in experiment 3 were irradiated at higher temperatures than 1 or 2 and so, as is the case, would be expected to have a lower frequency of one hit chromosome breaks.

As seen from table 2 there was no significant difference between means of chromosome breaks in the three groups at the 95% level. The high mean of the " continuous " dosage group even though not significantly higher, could be explained by the partial two-hit component in the chromosome break class.

DISCUSSION

The data presented completely verify the findings and conclusions of SAX (1939, 1940 and 1941) and SAX and LUIPPOLD (1952). In Tradescantia most chromosome breaks, measured by the frequency of dicentrics and centric rings, or other two-hit aberrations, restitute or undergo reunion soon after they are produced (see also CATCHESIDE 1948). In Tradescantia microspores once reunion or restitution has occurred the breakage ends involved are permanently removed from the pool of open breaks in the nucleus still available for rejoining. The evidence does not support the contention of LANE (1951) that restitution and reunion are delayed for several or many hours.

LANE's hypothesis that when a dose of 360 r is divided into two equal fractions the first fraction produces temporary physiological effects on chromosomes that render them less liable than before to radiation breakage is not supported. According to LANE these effects are maximal near 4 hours after the first fraction and are greatly reduced by 8 hours. The recovery at 8 hours as shown by an increased frequency of centric reunions did not occur in our experiments nor in those of SAX and LUIPPOLD (1952). In our material reunion frequencies were very similar in all lots given fractional X-ray treatments with a trend for a slight reduction in reunion frequency as the time between fractions was increased from 4 to 24 hours. However, the differences using the chi square test were too small to be statistically significant.

Our base line for centric reunions lies slightly above the means for samples receiving fractional doses. Somewhat similar results were reported by SAX (1939 and 1940) and by LANE (1951), where his fractions were 2, 4 or 6 hours apart. In other reported experiments the base line has been slightly below the values from split-dose experiments (SAX 1941 and SAX and LUIPPOLD 1952). Chance alone may determine, in a particular experiment, whether the base line will be above or below the value of the fractional-treatment group. It seems evident that when the dose fractions are 4 to 24 hours apart the reunion frequencies are not significantly different from those of the base line. It thus appears that breakage ends produced by the first fraction of a dose do not participate in reunions with breakage ends produced 4 to 24 hours later. All previous investigations were in agreement on the lack of participation of breakage ends from the first fraction with 1 to 4 hours between.

SAX (1939 and 1941) and CATCHESIDE *et al.* (1946b) found chromatid breaks independent of irradiation intensity. In this study (table 2) chromosome breaks, even with a minor two-hit component, were homogeneous. This homogeneity supports the theory that one-hit breaks are independent of irradiation intensity or dose fractionation.

According to SAX and ENZMANN (1939), CATCHESIDE *et al.* (1946b), SAX (1947) and CATCHESIDE (1948) more breaks are produced at low temperatures during irradiation than at high temperatures. In part the chromosome break frequencies recorded in table 1 were lower at the higher temperatures during irradiation. Because dose intensity compensation was employed

to counter the temperature effect two-hit aberrations were essentially the same. Increased breakage caused by low temperature was found due to higher oxygen tension by GILES and BEATTY (1950). GILES, BEATTY and RILEY (1951) found that in the absence of oxygen fewer breaks were induced at low temperatures and more at higher temperatures. LANE's concern (1951 and 1952) about exactly controlled temperature after irradiation (with little emphasis on temperature during irradiation) seems partially unjustified. The effects of temperature shock shown by SAX (1937), LACOUR (1949), and CALDECOTT and SMITH (1952) at extreme high and low temperatures should be avoided. However between irradiation and fixation at normal room temperatures, nuclear sensitivity for *Tradescantia paludosa* microspores is constant for the period 2–5 days before metaphase of the spore nucleus according to SAX (1941), GILES and RILEY (1949) and evidence presented here. Slight differences in the rate of development will have little if any effect on break frequencies when the material is fixed on the third or fourth day after the irradiation treatments, thereby making exactly controlled temperatures after irradiation unnecessary.

SUMMARY

When Tradescantia microspores were irradiated with 360 r of X-rays, the frequency of centric reunions was consistently higher with a single "continuous" dose than with two 180 r doses separated by 4, 8, 12 and 24 hours. In the fractional dose series there was no significant difference in reunion frequency with increasing time intervals between fractions. This fractional series was not significantly different from the base line. The frequency of one-hit chromosome breaks appeared to be independent of dosage fractionation. These findings confirm the observations of SAX, CATCHESIDE and other workers. Conversely the evidence does not support the findings of LANE where reunions and chromosome breaks were low when two 180 r doses were separated by 4 hours and were high when separated by 8 hours. The majority of previous investigations and the data presented here agree with the theory that Tradescantia chromosome breaks restitute or undergo reunion soon after their production. It is also apparent that restituted breaks rarely if ever reopen.

LITERATURE CITED

CALDECOTT, RICHARD S., and LUTHER SMITH, 1952 The influence of heat treatments on the injury and cytogenetic effects of X-rays on barley. Genetics **37**: 136–157.

CATCHESIDE, D. G., 1948 Genetic effects of radiations. Adv. Genetics **2**: 271–358.

CATCHESIDE, D. G., D. E. LEA and J. M. THODAY, 1946a Types of chromosome structural change induced by the irradiation of Tradescantia microspores. J. Genet. **47**: 113–136.

1946b The production of chromosome structural changes in Tradescantia microspores in relation to dosage, intensity and temperature. J. Genet. **47**: 137–149.

FABERGÉ, A. C., 1940 An experiment on chromosome fragmentation in Tradescantia by X-rays. J. Genet. **39**: 229–248.

GILES, N. H., and H. P. RILEY, 1949 The effect of oxygen on the frequency of X-ray-induced chromosomal rearrangements in Tradescantia microspores. Proc. Nat. Acad. Sci. **35**: 640–646.

GILES, N. H., and A. V. BEATTY, 1950 The effect of X-irradiation in oxygen pressure on chromosome aberration frequency in Tradescantia microspores. (Abstr.) Genetics **35**: 666–667.

GILES, N. H., A. V. BEATTY and H. P. RILEY, 1951 The relation between the effects of temperature and of oxygen on the frequency of X-ray-induced chromosome aberrations in Tradescantia microspores. (Abstr.) Genetics **36**: 552–553.

LACOUR, L. F., 1949 Nuclear differentiation in the pollen grain. Heredity **3**: 319–388.

LANE, G. R., 1951 X-ray fractionation and chromosome breakage. Heredity **5**: 1–35.

1952 Remarks on Sax and Luippold. Heredity **6**: 131–132.

LEA, D. E., and D. G. CATCHESIDE, 1942 The mechanism of the induction by radiation of chromosome aberrations in Tradescantia. J. Genet. **44**: 216–245.

SAX, KARL, 1937 Effect of variations in temperature on nuclear and cell division in Tradescantia. Amer. J. Bot. **24**: 218–225.

1939 The time factor in X-ray production of chromosome aberrations. Proc. Nat. Acad. Sci. **25**: 225–233.

1940 An analysis of X-ray-induced chromosomal aberrations in Tradescantia. Genetics **25**: 41–68.

1941 Types and frequencies of chromosomal aberrations induced by X-rays. Cold Spring Harbor Symp. Quant. Biol. **9**: 93–101.

1947 Temperature effects on X-ray-induced chromosome aberrations. Genetics **32**: 75–78.

SAX, KARL, and E. V. ENZMANN, 1939 The effect of temperature on X-ray-induced chromosome aberrations. Proc. Nat. Acad. Sci. **25**: 397–405.

SAX, KARL, and H. LUIPPOLD, 1952 The effect of fractional X-ray dosage on the frequency of chromosome aberrations. Heredity **6**: 127–131.

SNEDECOR, G. W., 1946 Statistical methods. Iowa State College Press. Ames, Iowa. 485 pages.

STUDIES ON KAPPA-LIKE PARTICLES IN SENSITIVES OF PARAMECIUM AURELIA, VARIETY 4 [1]

EARL D. HANSON [2]

Received August 20, 1953

KILLER paramecia are resistant to the effects of the poison, paramecin, which they produce. Paramecia susceptible to this same poison are called sensitives (SONNEBORN 1939, 1943). The killer character in *Paramecium aurelia* depends on the presence both of the *K* gene in the nucleus and of the self-reproducing, mutable, microscopically visible particles, called kappa, in the cytoplasm, whose presence is correlated with the production of paramecin (PREER 1946, 1948b; SONNEBORN 1946). The replacement of the *K* gene of killers by its recessive allele, *k*, causes the disappearance of kappa from the cytoplasm with a concomitant loss of killing ability, which results, finally, in sensitive lines which do not possess kappa (SONNEBORN 1943). The loss of kappa from a variety 4 killer, whether due to genetic, as just mentioned, or environmental reasons, results in the transformation of that animal into a sensitive whose progeny are all sensitive (WILLIAMSON, JACOBSON and STOCK 1952; PREER 1948a; SONNEBORN 1946; VAN WAGTENDONK, CONNOR and MILLER 1953).

The sensitive, non-killer character thus results from the absence of kappa. It is also known to occur when the number of kappa particles per cell is low (SONNEBORN 1946). According to estimates by SONNEBORN (1946), PREER (1948a), and CHAO (unpublished) the cells are sensitive if there are less than about 20 kappa particles in it. The present paper reports a new type of sensitive which arose unexpectedly from killer ancestors. Curiously, it has the genotype and seems to have the cytoplasmic condition proper to a killer, i.e., it carries gene *K* and large numbers of cytoplasmic kappa-like particles. Such an unprecedented constitution for a sensitive obviously leads to important modifications of the present views of the killer-sensitive system. These will be discussed after presenting an account of the origin and genetic analysis of this sensitive and a preliminary report on the behavior of kappa and the cytoplasmic particles of the new sensitive when both are in a common cytoplasm.

MATERIALS AND METHODS

Animals of *Paramecium aurelia*, varieties 2, 4 and 8, were used in the work reported here. The variety 4 animals are: stock 51, the standard killer stock

[1] Contribution No. 536 from the Zoology Department of Indiana University. The work of the author is in partial fulfilment of the requirements of the Ph.D. degree.

[2] During the course of this investigation the author was a Predoctoral Fellow of the National Science Foundation. This work was aided in part by a grant from the Rockefeller Foundation for work in Genetics at Indiana University, and in part by a grant-in-aid to PROF. T. M. SONNEBORN from the American Cancer Society upon the recommendation of the Committee on Growth of the National Research Council.

(SONNEBORN 1943); stock d186, a derived stock containing the *k* gene from stock 29 but otherwise essentially isogenic with stock 51 (DIPPELL 1950); stock 47, a killer; and stock 139, isolated as a killer from nature but now a sensitive. All of these variety 4 stocks are interfertile giving viable F_1 and F_2 progeny. Stocks of variety 2 were used only as killer testers. Stock 31, variety 8, was used only as a standard sensitive tester. The killing action of stock 51 (DIPPELL 1950; SONNEBORN 1943), stock 47 (DIPPELL 1950), and the variety 2 killers (PREER 1948a) has already been described.

The methods used for maintaining the cultures, making the crosses, inducing autogamy, setting killer tests, and obtaining specific antisera are those set forth by SONNEBORN (1950).

ORIGIN, DESCRIPTION AND GENETIC ANALYSIS OF THE SENSITIVES

Origin. Normally the F_2 derived by autogamy from the cross between two homozygous killer parents are all killers. However, in one such experiment, the F_2 consisted of two sensitives and 122 killer clones. In this experiment both parents were killers homozygous for gene *K*. One parent was stock 51,

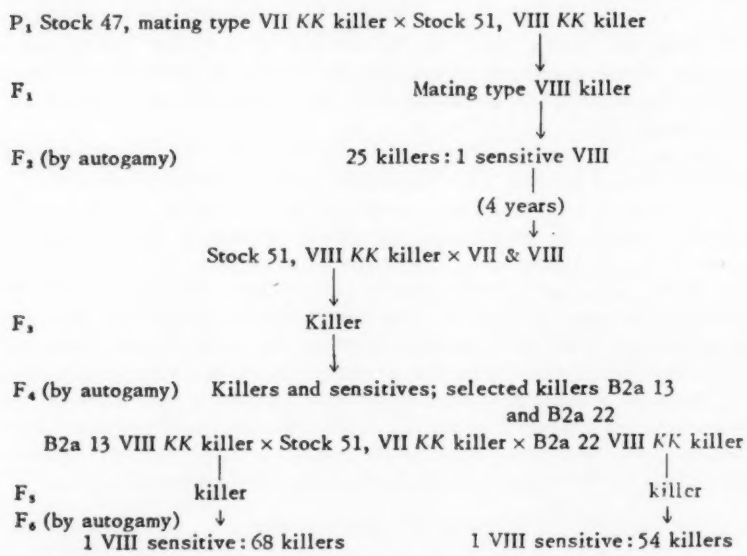
P1 Stock 47, mating type VII KK killer × Stock 51, VIII KK killer
F1
Mating type VIII killer
F2 (by autogamy)
25 killers : 1 sensitive VIII
(4 years)
Stock 51, VIII KK killer × VII & VIII
F3
Killer
F4 (by autogamy)
Killers and sensitives; selected killers B2a 13 and B2a 22
B2a 13 VIII KK killer × Stock 51, VII KK killer × B2a 22 VIII KK killer
F5
killer
killer
F6 (by autogamy)
1 VIII sensitive : 68 killers
1 VIII sensitive : 54 killers

FIGURE 1.—Diagram showing genetic history of the two exceptional sensitives.

mating type VII, and the other was mating type VIII but of hybrid origin, as set forth in figure 1. The two killers B2a 13 and B2a 22, which were taken from the F_4, are the ones used for the cross to stock 51. It was in the F_6 that the exceptional sensitives were found. Another unexpected sensitive was the one found in the F_2. The analysis of this line will be reported elsewhere and

is thought not to have any bearing on the present case for reasons to be brought out later.

Phenotype. The diagnosis of these two exceptional cultures as sensitives is based on two facts: they are killed when mixed with stock 51 killers or with breis of these killers; and they and their breis cannot kill standard sensitive cultures.

The action of killers other than stock 51 killers has also been tested on these animals. The reason for testing these other killers was that DIPPELL (1950) has found mutations of stock 51 kappa which result in the mutant killers being resistant to their own paramecin but not to that of the original 51 animals. It was conceivable that in the special sensitives being considered here, there had been two mutations; one which changed the type of killing action and therefore also the type of resistance, and then a second which eliminated the ability to kill. The final result being animals unable to kill stock 51 and at the same time being sensitive to it, but retaining, perhaps, a resistance to some other kind of paramecin. However, in no case was any resistance detected. These sensitive lines are susceptible to the paramecin produced by the following killers: stock 51 and stocks G, 36 and 308-2 of variety 2. It was found that the sensitive lines were killed in the manner typical for each type of killer.

Genotype. A priori it was conceivable that these lines had become sensitive to the action of paramecin due to a nuclear change, e.g., mutation of the *K* gene to its recessive allele, *k*, which cannot support kappa. One such case is already known (HANSON unpublished). This is the exceptional case mentioned earlier and represented by the sensitive found in the F_2 (fig. 1). Breeding analyses were carried out to test this hypothesis in the present case.

Each sensitive was crossed to a known standard pure killer. Stock 51 was used as this killer. If the sensitive parent lacked gene *K* or any other gene necessary for the maintenance or expression of the killer trait, the F_1 should be heterozygous at this locus and the F_2 obtained by autogamy should segregate killers and sensitives in a 1 : 1 ratio. On this assumption the crosses were followed through to the F_2. The results, summarized in table 1, show that no segregation occurred in the F_2; all of the F_2 were killers and of the standard stock 51 type. The marked difference in results when a stock with a known

TABLE 1

Summary of the results of crossing the sensitives of anomalous origin to stock 51. (Symbols: (K) = killer, (S) = sensitive)

Cross	Number of F_2 clones obtained	Results					
		Observed		Theoretical			
				Single gene difference		No gene difference	
		(K)	(S)	(K)	(S)	(K)	(S)
Sensitive line A × Stock 51	120	120	0	60	60	120	0
Sensitive line B × Stock 51	95	95	0	47.5	47.5	95	0
Stock d186 × Stock 51	116	60	56	58	58	116	0

single gene difference (*kk* instead of *KK*) is similarly crossed to stock 51 is also shown in table 1; here a 1:1 segregation of killers and sensitives does occur in the F_2. This cross serves as a control on the experimental cross. Hence, it may be concluded that the sensitives do not differ from the standard killers in any gene affecting the killer trait.

Cytoplasm. With the elimination of the possibility of a relevant nuclear mutation in the origin of the sensitive clones, attention was directed to the cytoplasm. As a working hypothesis it was assumed that kappa had, for undetermined reasons, been lost from these animals. This hypothesis was tested by staining the lines in question using DIPPELL and CHAO's modification of DELAMATER's basic fuchsin technique (SONNEBORN 1950), and examining them for the presence or absence of kappa particles.

The results of this cytological study were quite unexpected: kappa-like particles were found in the cytoplasm of these animals. A preliminary estimate would place the number of these particles as about 400 per cell.

Using the techniques of phase microscopy developed by PREER and STARK (1953) it has been found that the shape of these particles differs from that of kappa. According to PREER and STARK (1953), kappa in variety 4 consists of essentially two types of particles: (1) subspherical or ovoid bodies averaging 1.2 micra in length, either single or, less commonly, double—a long "single" with a median constriction; (2) larger and more irregularly shaped particles which may or may not contain a refractile body. The particles found in the sensitives do not show this dimorphism. These particles are invariably rod-shaped; they appear as singles, doubles, even triples, and, rarely, quadruples. No refractile bodies have ever been observed in them. In lines fed to multiply at 0.5 fissions per day, the size of single particles in a sample of 30 varied from 1.1 micra to 5.4 micra, with a mean value of 2.7 micra. The compound forms may be as large as 10 micra. These observations were made under bright phase (American Optical bright H objective) at a magnification of 1455×.

PREER and STARK (1953) have reported the possibility of serological studies on kappa in breis made from killers. In making these studies whole animals are broken up on a glass slide in a drop of the undiluted testing serum and then observed under bright phase. Using this method it has been possible to demonstrate a serological difference between kappa and the particles in the sensitives. Using antisera prepared against stock 51 killers, against stock 29 which contains no kappa-like particles, and against the particle-containing sensitives, it was found that kappa was agglutinated only in the presence of the anti-killer serum, and the new particles only in the presence of their specific antiserum. This agglutination takes place rapidly: in a matter of minutes the particles stick to each other in a random manner, regardless of whether they are singles, doubles or of larger size.

From the observations reported above it is clear that kappa and these newly found particles differ in a number of respects, such as size, shape, serologic properties, and paramecin production. Because of these differences the new particles have been provisionally designated "pi" in contradistinction to the

already familiar kappa. The implications of this designation are meant to be twofold. (1) Because of the differences just cited, the origin of pi as a mutant kappa may be doubted, a point that will be discussed more fully later. Therefore the terminology used by DIPPELL (1950) for kappa mutants has not been used. (2) These pi particles are unique in two respects in the killer-sensitive system of *P. aurelia*. First, the high concentration of the particles in each animal eliminates the possibility that these animals are sensitives as a result of a low kappa concentration (SONNEBORN 1946). Second, the lack of any detected killing ability and the lack of resistance to any killer, differentiates pi sensitives from DIPPELL's 51m5 mutant which showed little or no killing ability but yet was resistant to stock 51 paramecin.

Relation of pi to the genetic constitution of the paramecia. An experiment was designed and carried out to test the possibility that pi, like kappa, is dependent on the *K* gene for its continued survival. The test was to cross the pi-bearing sensitives to *kk* sensitives, to obtain autogamy in the pi-bearing F_1, and to ascertain whether the F_2 generation thus obtained segregates 1 : 1 for the capacity to maintain pi. If so, then each F_2 culture would have to be analyzed by crosses to discover whether the cultures that lost pi are homozygous for gene *k*, while those that retained pi are homozygous for gene *K*.

The details of this analysis are as follows: The *kk* sensitive stock employed was d186. It was crossed to a pi-bearing sensitive (*KK*), marked phenotypically by a different serotype from that manifested by d186. The pi-bearing F_1, as determined by its serotype and confirmed in two cases by staining for pi, was used to obtain an F_2 generation by autogamy. Each of 47 cultures was (1) stained to ascertain whether pi was present or not; and (2) crossed to pure killers (*KK*) of stock 51 to determine by the character of the F_2 obtained from the killer F_1, whether the tested cultures carried *K* or *k*. In this second test, as in the experiments set forth above (page 233), each tested culture that carried gene *K* would yield only killers in the F_2. The data of the entire analysis are summarized in table 2, which shows that pi, like kappa, is maintained only in the presence of the *K* gene. All 22 of the F_2 cultures shown to carry *K* also carried pi; all 25 shown to carry *k* lacked pi.

The only observation which interferes with the decisiveness of the experiment is that in three of the lines homozygous for the *k* gene, staining by the basic fuchsin technique revealed a small number of animals containing particles similar in size and number to pi. The reason for the presence of these particles is obscure. They are in all probability not due to the slower loss of particles in these lines as compared to their sister lines because (1) the concentration appears to be essentially normal, and (2) because, using kappa loss (CHAO 1953) as a parallel, the particles should not have persisted beyond the 14th fission after the animals became homozygous for gene *k*, and these lines were past their 40th fission. A possible explanation is that a few of the descendants of a single autogamous isolation regenerated their macronucleus from a fragment of the previous macronucleus (SONNEBORN 1945a, 1945b). The regenerated macronucleus would of course, in these crosses, be heterozygous at the *K*

TABLE 2

Summary of the results from the genetic and cytological analysis of a pi containing culture to determine whether or not pi needs the K gene for its continued presence in an animal.

Cross	Total number of F_2 clones	Results (K)	Results (S)
Experimental:			
Pi sensitive × Stock d186	54	0	54
Controls:			
Pi sensitive × Stock 51 killer	137	137	0
Stock d186 × Stock 51 killer	127	67	60

Of the 54 lines of sensitives from the experimental cross above, 47 were analyzed genetically for the presence of the K or k gene, and cytologically for the presence or absence of pi.

	Genetic results	
	Number of crosses showing 1 : 1 segregation in F_2	Number of crosses showing no segregation in F_2
Each sensitive clone crossed to Stock 51 killer	25	22
	Cytological results	
	Lines not showing pi	Lines showing pi
Same sensitive clones	25	22

In each of the lines carrying the K gene pi was also found; in each of the lines carrying the k gene no pi was found. (See text for temporary exceptions.)

locus and could still maintain pi in the paramecium in spite of the presence of a *kk* micronucleus (SONNEBORN 1946b). Since no killing would occur in these cultures, animals with and without pi would both survive. Pi would, however, be lost whenever an animal containing it would undergo a normal autogamous nuclear reorganization, as this would cause the macronucleus containing the *K* gene to be replaced by a new macronucleus not containing it. In line with this interpretation is the fact that a considerably later repetition of the staining, using PREER's (unpublished) crystal violet technique was used on these same lines and no evidence of particles was found.

Mixed cytoplasms. Of especial interest is the question of what happens when cytoplasm is exchanged during conjugation (SONNEBORN 1950) between pi and kappa containing animals. Repeated observations show that both exconjugants produce killer cultures by the eighth post-conjugational fission, the culture from the former sensitive member of the pair gradually transforming into a killer, and the culture from the killer member simply maintaining its original killer character. Furthermore, if these exconjugant clones are carried along by continual subculturing either by daily isolation of single animals or of small random samples, dead animals are found in the cultures when they

are allowed to starve down. No death has been observed in the well-fed cultures. The amount of death varies from clone to clone. The death appears to be due to the action of paramecin since the corpses have the appearance of well-starved sensitives that have been exposed to stock 51 paramecin.

One explanation for the origin of these " sensitives " was thought to lie in the fact that since pi was known to have replaced kappa once (when pi was first found), that it therefore was conceivably doing it again with the result that the sensitives were being killed off, as fast as they appeared, by the remaining killers in the culture. To test this possibility numerous isolations were made both before and after the mortality set in, with the intent of isolating, before it was killed, an animal that was to become a sensitive. None of these isolations ever established pure sensitive lines, though they did continue to throw dead animals. Even repeated selection from the cultures showing the greatest amount of this autolethality did not yield a single sensitive culture.

Stock 139. Stock 139 when first brought into the laboratory from nature was a killer. All the cultures of this stock which are now on hand are sensitives. Cytologic examination of this stock shows that pi-like particles are also present. The exact history of the change from killer to sensitive is not known for these animals.

DISCUSSION

Origin of pi

Two explanations of the origin of pi need to be considered: that pi is a mutant kappa; and that pi represents an independent infection from an exogenous source.

Mutation of kappa. DIPPELL (1950) has already reported that stock 51 kappa can mutate. However, the mutations analyzed by DIPPELL were in all cases changes in the killing character of the mutants, i.e., the original " hump " type killers became either " spinner " killers or very weak hump killers that were still resistant to their own type of paramecin. PREER (1948a) also described a variety 2 mutant, Gm1, which shows a different type of killing than that manifested by the original stock G killer. Mutations from killer to sensitive could be detected only under certain conditions. The sensitives would have to be isolated from the killer culture before they were affected by the killers, for otherwise they would be lost. But they must not be isolated so early that the resulting culture would produce, by a chance segregation at fission of killer and non-killer particles to the daughter cells, any paramecia that contained only the non-mutated particles, as this would reestablish a pure killer line. It will be recalled that these conditions could have been realized when the pi-bearing lines were first discovered because both pi lines were isolates from killer cultures. From these considerations it is not unreasonable to assume, *a priori*, that paramecin-producing kappa might mutate to a non-paramecin-producing form which could be detected if the unmutated kappa were replaced by the mutated kappa, and if the animal containing it were isolated from the killer culture at the appropriate time.

Three lines of evidence supporting this mutation hypothesis can be offered. First, the pi lines were derived from kappa-bearing lines. Second, pi, like kappa, needs the *K* gene for its continued maintenance. Third, pi and kappa are demonstrable by the same cytological techniques.

There are, however, certain facts which are not readily reconciled with the mutation hypothesis, though they are not fatal to it. From the description of pi it can be seen that there are certain points of dissimilarity between these particles and kappa. Pi does not produce paramecin, animals possessing pi are not resistant to any tested paramecin, pi is morphologically and serologically distinguishable from kappa, and, lastly, there is some difference, as yet unknown, which determines an apparent mutual exclusion effect when pi and kappa are in the same animal, such that in one instance (that of the original discovery of pi) kappa was replaced by pi, but in other instances, after cytoplasmic exchange occurs between pi- and kappa-bearing animals during conjugation, only killer animals have been recovered. These multiple differences can be explained by any one of three different postulates. (1) The kappa mutation that gave rise to pi is pleiotropic in its effect; (2) there has been an accumulation of different mutations in the particles, including one that eliminated the killing ability and resistance of the animals; and (3) a combination of points (1) and (2). To distinguish clearly between these alternatives will be difficult. However, on the basis of the second and third postulates, one would expect that, if enough different characteristics of the particles were studied, differences would be detected between particles of different origin due to the fact that unlike mutations have probably accumulated in the different lines. If no differences are found the first alternative would be indicated. Preliminary comparison of pi particles in stock 139 with those in the lines reported here show no significant differences.

Infection from an exogenous source. The reasons for entertaining this hypothesis as a possible explanation for the origin of pi are: if kappa is a "viroid" or a relative of the green alga of *P. bursaria* as ALTENBURG (1946) suggests, or if kappa can be compared with Rickettsiae as PREER (1950) has suggested, then pi, also, could be of extrinsic origin and from a source different than kappa.

The critical investigation is to determine whether pi appears only in lines which carry kappa. In the cases known thus far pi has been found only under such conditions. This is true not only for the two lines analyzed in this paper, but also for stock 139, and for certain lines examined by PREER, SIEGEL, and STARK (1953). The latter have found pi-like particles in certain of the mutant killers of stock 51 earlier analyzed by DIPPELL (1950). Yet to be discovered is whether pi ever exists in lines known not to have contained kappa. In the light of our present knowledge, therefore, the mutation hypothesis is thought to be the more likely explanation for the origin of pi.

Pi and kappa in a common cytoplasm

If it can be assumed that there is no intracytoplasmic selection for either pi or kappa, that on the average they both multiply at the same rate and that new

origins of pi are so rare as to be statistically negligible—in sum then, that random segregation is the only factor governing the distribution of these particles, then the replacement of kappa by pi on this basis would be a rare but possible event. However, there are two points which indicate that random segregation is not the only factor involved here.

First, in a cross such as described in the mixed cytoplasm work, two different situations conceivably exist in the respective exconjugants of a single pair. At the start the killer member of the pair possesses an excess of kappa over pi in its cytoplasm, and in the sensitive member the reverse holds true. The probability of chance segregation alone yielding any pure pi sensitives (lacking kappa) from the killer clone would be low, just how low depending inversely on the amount of cytoplasm exchanged. And conversely, in the clone derived from the sensitive parent, the probability of getting cytoplasms pure for kappa on this same basis would be similarly low. Which is to say, if we isolate the members of the clone derived from the sensitive and avoid selection due to the action of paramecin, we should find a preponderance of sensitive as compared to killer subcultures. But this is not the case. As stated previously, what is found is all killer subcultures. The first point, then, is that random segregation of the two types of particles is not apparent under the conditions described here.

The other point is that in the exconjugant clones corpses are found. The dead animals appear to have died as a result of the action of paramecin, and presumably then, they were sensitives. If these sensitives are due to the replacement of kappa by pi, as has already been suggested, and if this replacement is dependent on random segregation alone, then it should be possible to isolate pi-sensitive lines from these cultures. For example, if in a given culture 10% of the animals were dying, then isolation of 100 animals from this culture at the appropriate time should yield roughly 10 lines of pi-containing sensitives. But as has already been reported, contrary to expectations, no sensitive lines have ever been established. This is also taken to indicate that random segregation of these particles is not solely responsible for their distribution.

In this connection should be mentioned the observation made by DIPPELL (1950) that the normal stock 51 kappa—"hump" kappa—when in the same cytoplasm as either "spinner" or "resistant" kappa (both of these latter are mutations of hump kappa) does not result in the appearance of any dead animals in such cultures. DIPPELL, therefore, concludes that a sort of population equilibrium is set up within such animals as regards the kappa they contain, such that both types persist within a single animal. But it is admitted that this equilibrium concept does not readily explain how both of these mutants were established spontaneously as pure mutant cultures lacking completely the hump kappa.

The nature of the other factors involved in the distribution of pi and kappa is not known. They may be concerned with different rates of multiplication of the two types of particles, which may in turn depend on the competition for a common precursor, e.g., the *K* gene product. Or again, it may simply be due to as yet unidentified factors in the culture technique which may or may not

affect the two possibilities first suggested. These speculations are now under experimental analysis in an attempt to obtain additional information which will throw light on the processes involved in the establishment, maintenance, and transmission of these materials of cytoplasmic heredity.

SUMMARY

A new type of sensitive has been found in the killer-sensitive system of *Paramecium aurelia*. This sensitive contains in its cytoplasm a type of particle not encountered before in this organism. The particles, referred to as pi, are thought to be mutant kappa particles. They arose from a killer culture; they are dependent on the *K* gene for their survival; and they possess the same staining capacities as kappa. Certain points of dissimilarity between pi and kappa are also described and discussed. The present extent of the knowledge of the interrelationships between pi and kappa, when both are in a common cytoplasm, are presented and briefly discussed.

ACKNOWLEDGMENT

The author wishes to thank DR. T. M. SONNEBORN for his invaluable criticisms and encouragement throughout the course of this work.

LITERATURE CITED

ALTENBURG, E., 1946 The symbiont theory in explanation of the apparent cytoplasmic inheritance in Paramecium. Amer. Nat. **80:** 661–662.

CHAO, P. K., 1953 Kappa concentration per cell in relation to the life cycle, genotype and mating type in *Paramecium aurelia*, variety 4. Proc. Nat. Acad. Sci. **39:** 103–113.

DIPPELL, R. V., 1950 Mutation of the killer cytoplasmic factor in *Paramecium aurelia*. Heredity **4:** 165–187.

PREER, J. R., 1946 Some properties of a genetic cytoplasmic factor in Paramecium. Proc. Nat. Acad. Sci. **32:** 247–253.

1948a A study of some properties of the cytoplasmic factor, "kappa," in *Paramecium aurelia*, variety 2. Genetics **33:** 349–404.

1948b Microscopic bodies in the cytoplasm of "killers" of *Paramecium aurelia* and evidence for the identification of these bodies with the cytoplasmic factor, kappa. Genetics **33:** 625.

1950 Microscopically visible bodies in the cytoplasm of the "killer" strains of *Paramecium aurelia*. Genetics **35:** 344–362.

PREER, J. R., and P. STARK, 1953 (in press).

PREER, J. R., R. W. SIEGEL and P. STARK, 1953 (in press).

SONNEBORN, T. M., 1939 *Paramecium aurelia*: mating types and groups, lethal interactions; determination and inheritance. Amer. Nat. **73:** 390–413.

1943 Gene and cytoplasm. I. The determination and inheritance of the killer character in variety 4 of *P. aurelia*. Proc. Nat. Acad. Sci. **29:** 329–338.

1945a Gene action in Paramecium. Ann. Mo. Bot. Gdn. **32:** 213–221.

1945b The dependence of the physiological action of a gene on a primer and the relation of primer to gene. Amer. Nat. **79:** 318–339.

1946a Experimental control of the concentration of cytoplasmic genetic factors in Paramecium. Cold Spring Harbor Symp. Quant. Biol. **11:** 236–255.

1946b A system of separable genetic determiners in the cytoplasm of *Paramecium aurelia*, variety 4. Anat. Rec. **94:** 346.

1950 Methods in the general biology and genetics of *Paramecium aurelia*. J. Exp. Zool. **113**: 87–148.

SONNEBORN, T. M., W. JACOBSON and R. V. DIPPELL, 1946 Paramecin 51, an antibiotic produced by *Paramecium aurelia*: amounts released from killers and taken up by sensitives, conditions protecting sensitives. Anat. Rec. **96**: 18–19.

VAN WAGTENDONK, W. J., R. E. CONNOR and C. MILLER, 1953 (in press).

WILLIAMSON, M., W. JACOBSON and C. C. STOCK, 1952 Testing of chemicals for inhibition of the killer action of *Paramecium aurelia*. J. Biol. Chem. **197**: 763–770.

CYTOGENETIC EFFECTS OF X-RAY AND THERMAL NEUTRON IRRADIATION ON SEEDS OF BARLEY [1]

RICHARD S. CALDECOTT, B. H. BEARD AND C. O. GARDNER [2]
University of Nebraska [3] *and Brookhaven National Laboratory* [4]

Received August 24, 1953

IN an earlier paper (CALDECOTT *et al.* 1952) it was demonstrated that there were pronounced differences in the biological effect of X-rays and thermal neutrons on dormant seeds of barley. The present investigation was undertaken in an effort to elucidate these differences and attempt to explain them on purely physical assumptions.

The reader is referred to reviews by LEA (1947) and SPARROW and RUBIN (1952) and a paper by CONGER and GILES (1950) for discussions pertaining to the subject.

MATERIALS AND METHODS

Dormant seeds of Himalaya Barley (C.I. 620) were used for the entire study. The seeds originated from the progeny of a single plant in 1947 and thus would be expected to have a high degree of genetic uniformity. They were stored in a humidity-controlled desiccator for four to six weeks prior to treatment.

In the major part of the investigation, conducted in 1952, samples of the dormant seed were subjected to various doses of either X-rays or thermal neutrons (see table 1). The X-radiation was filtered through 1 mm of aluminum and was administered to the seeds as they lay in a single layer in an uncovered Pyrex Petri dish on a rotating turntable. The radiation facility was operated at 250 KVP and 30 ma giving approximately 800 r per minute at the surface of the seeds as measured with a Victoreen Integron. The highest X-ray dose was given in approximately 31 minutes.

For treatment with thermal neutrons the seeds were placed in a single layer in a lucite container and exposed in the thermal column of the nuclear reactor at the Brookhaven National Laboratory. Dosage determinations were obtained by exposing gold foils with the seeds and relating the extent of induced radioactivity in the foils to the flux of thermal neutrons in the column. The highest dose used was administered in approximately 10 hours. Essentially, all other doses required proportionately less time. The cadmium ratio in the thermal

[1] Published with approval of the Director as paper No. 619, Journal Series, Nebraska Agricultural Experiment Station.

[2] Associate Radiobiologist, Brookhaven National Laboratory and Research Assistant and Associate Professor, University of Nebraska, respectively.

[3] Research conducted under Contract No. AT(11-1)-169, Atomic Energy Commission.

[4] A portion of the research reported herein was carried out at the Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

TABLE 1

Effect of X-rays and thermal neutrons on dormant seeds of barley as measured by survival to maturity.

Treatment	No. seeds planted	Survival	
		Number	% (of control)
Control (no treatment)	3000	2537	100
Dose of X-rays			
5000 r	1500	1218	96.0
10000 r	1500	913	72.0
15000 r	1500	846	66.7
20000 r	1500	806	63.5
25000 r	1500	430	33.9
Dose of thermal neutrons per cm^2			
2.3×10^{12}	1000	881	104.2
3.0×10^{12}	1000	789	93.3
4.1×10^{12}	1000	840	99.3
5.5×10^{12}	1000	806	95.3
6.5×10^{12}	1000	896	106.0
7.5×10^{12}	1000	819	96.8
11.5×10^{12}	1000	847	100.2
12.2×10^{12}	1000	727	86.0
14.2×10^{12}	1000	784	92.7
15.5×10^{12}	1000	328	38.8
20.0×10^{12}	1000	371	43.9
22.2×10^{12}	1000	0	0.0
23.0×10^{12}	1000	523	61.8
30.0×10^{12}	1000	0	0.0
30.1×10^{12}	1000	0	0.0

column was about 5000 : 1 and the gamma contamination approximately 50 r per hour.

After treatment the seeds (see table 1 for the number given each treatment) were space planted and grown under irrigation in a nursery at the University of Idaho Experimental Station in Aberdeen, Idaho, during the spring and summer of 1952.

At microsporogenesis spikes were removed from a random sample of the plants surviving each treatment and analyzed to determine the frequency of interchanges detectable at first metaphase. Only one spike was removed from any one plant.

At maturity a minimum of two and a maximum of four of the earliest ripening spikes were removed from each plant and placed in an envelope. They were then shipped to Lincoln, Nebraska, where seedling mutation studies were conducted after the method of STADLER (1930). Spikes which shattered during shipment were discarded.

All cytological analyses were made using the acetocarmine smear technic. Carnoy's solution (6 parts 95 percent ethyl alcohol : 3 parts chloroform : 1 part glacial acetic acid) was used for fixing and storing the specimens.

Because of the uncertainties in neutron dosimetry, as reflected by inconsistencies in table 1, a subsidiary experiment was conducted in 1953 to verify the growth and survival data obtained under field conditions in 1952. The

seed source was the same for both experiments and the radiations were conducted in a similar way.

In the 1953 study samples of 120 seeds were subjected to various doses of X-rays and thermal neutrons. Each sample received one of the following doses: X-rays 5,000 r, 10,000 r, 15,000 r, 20,000 r, 25,000 r, 30,000 r, 35,000 r, 40,000 r, 45,000 r and 50,000 r; thermal neutrons 1, 2, 3, 4, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½ and 10 hours at a flux approximating $7.0 \times 10^8 N_{th}/cm^2/sec$. For the thermal neutron treatment, in contrast to the 1952 experiments where the seeds were exposed at different times and the gold foil method was used for dose determinations, in 1953 the seeds were placed in the thermal column at the same time and 120 were removed at each of the time intervals designated. By using this procedure it was assured that each of the treated lots received a different dose of thermal neutrons and there could be no overlapping of doses between seeds removed at different times. This method of handling the material was used because it was recognized that the errors involved in gold foil dosimetry may be of a magnitude such as to render meaningless slight differences in absolute calculated values.

After irradiation 70 seeds were germinated and those which survived were grown to maturity in plant bands in steam sterilized soil. The other 50 seeds were germinated and grown in Petri dishes for seedling height studies.

EXPERIMENTAL RESULTS

Growth and survival

As indicated earlier (CALDECOTT *et al.* 1952), for any dose sufficient to induce a significant reduction in seedling height, seedlings from seeds subjected to thermal neutrons are much more uniformly affected than seeds subjected to X-radiation. This uniformity of response was apparent in the present study at all stages of plant development in greenhouse, laboratory, and field experiments. It is best exemplified in figures 1 to 4 where it is evident from the appearance of the seedlings in the first two figures and from the plots of survival as a function of dose in figures 3 and 4. The data presented in these four figures are entirely from the 1953 study. All other data are from the experiments conducted in 1952.

The 1952 data for survival under field conditions lend support to the 1953 study (table 1). They show that, even under field conditions where the rigors of environment take a toll of weakened plants, for most doses of thermal neutron irradiation, the survival is about as good as for controls. It is considered likely that an error in dosimetry is the reason that a calculated dose of 22.2×10^{12} thermal neutrons per cm^2 was lethal to all seeds whereas 62% of the seeds given a calculated dose of 23.0×10^{12} thermal neutrons per cm^2 survived.

Frequencies of chromosomal bridges and fragments

The frequencies of dicentric bridges at anaphase of the first cycle of cell divisions in root tip cells were determined on samples of seed irradiated inde-

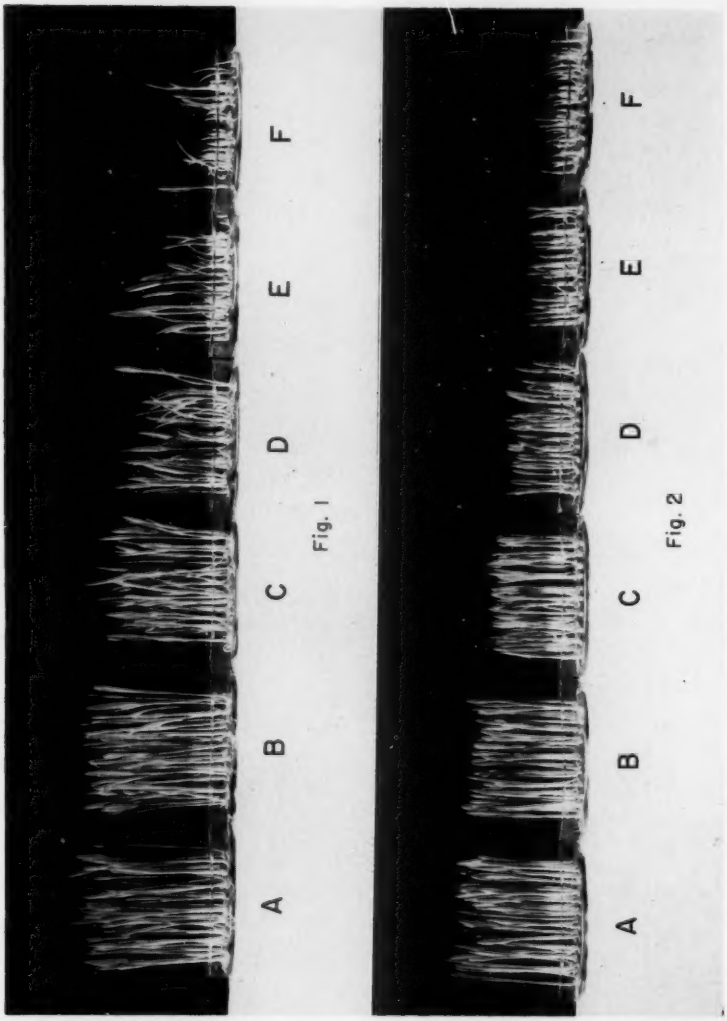

FIGURE 1.—Seven-day-old seedlings of barley from dormant seed treated with X-rays. A, Control (no treatment); B, 5,000 r; C, 15,000 r; D, 25,000 r; E, 35,000 r; F, 45,000 r.

FIGURE 2.—Seven-day-old seedlings of barley from dormant seed treated with thermal neutrons for the times designated at an approximate flux of 7.0×10^5 $N_{th}/cm^2/sec$. A, Control (no treatment); B, 2 hours; C, 4 hours; D, 6 hours; E, 8 hours; F, 10 hours.

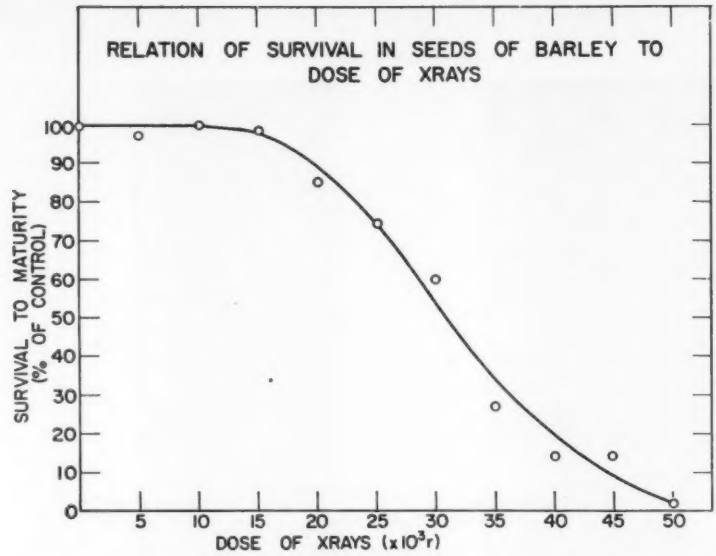

FIGURE 3.—Relation of survival in seeds of barley to dose of X-rays.

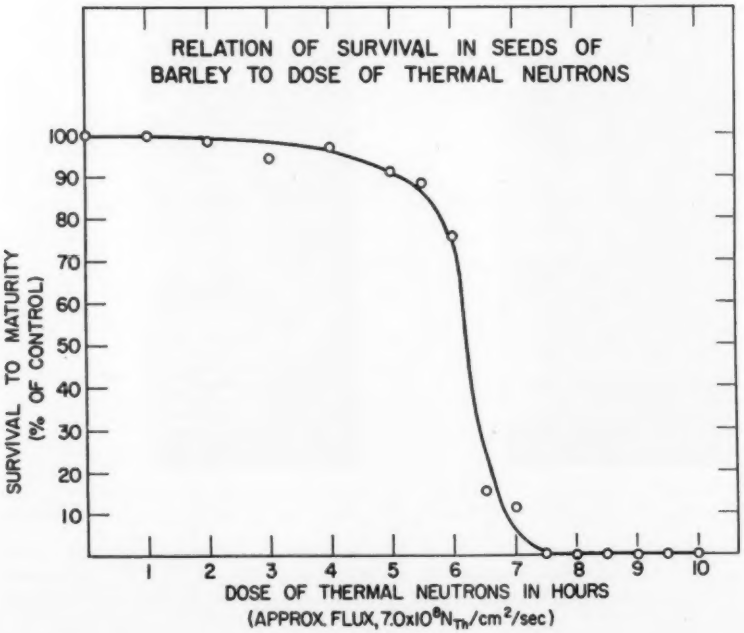

FIGURE 4.—Relation of survival in seeds of barley to dose of thermal neutrons.

pendently (but in a similar way) to the irradiations reported in table 1. These data were presented in tabular form in a report by CALDECOTT *et al.* (1952) and are presented graphically here for comparative purposes (fig. 5). It is of significance that these bridges usually occur in pairs and result from inter-

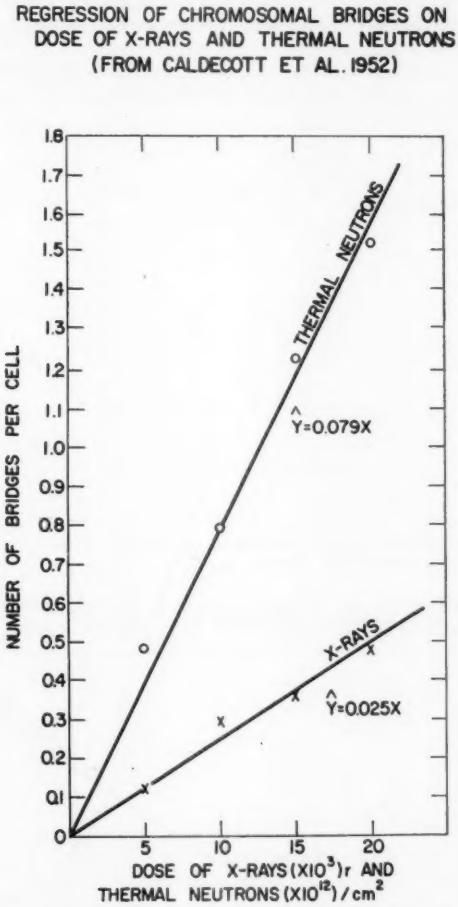

FIGURE 5.—Regression of chromosomal bridges on dose of X-rays and thermal neutrons. (From CALDECOTT *et al.* 1952.)

changes between two centric fragments and not from fusions between sister chromatids at a common point of breakage (see CALDECOTT and SMITH 1952b). From figure 5 it is obvious that the frequencies of these exchanges are directly proportional to the dose of both X-rays and thermal neutrons. These observations will be considered in the discussion because most workers have reported

TABLE 2

Effect of X-rays and thermal neutrons on dormant seeds of barley as measured by the frequency of interchanges in microsporocytes.[1]

Treatment	No. spikes examined	Interchanges observed	
		Total No.	Per 100 spikes
Control (no treatment)	173		
Dose of X-rays			
5000 r	451	40	8.9
10000 r	420	57	13.6
15000 r	401	60	15.0
20000 r	398	111	27.9
25000 r	196	66	33.7
Dose of thermal neutrons per cm^2			
2.3×10^{12}	151	15	9.9
3.0×10^{12}	150	29	19.3
4.1×10^{12}	150	50	33.3
5.5×10^{12}	149	40	26.8
6.5×10^{12}	150	50	33.3
7.5×10^{12}	150	61	40.7
11.5×10^{12}	150	88	58.7
12.2×10^{12}	150	86	57.3
14.2×10^{12}	150	95	63.3
15.5×10^{12}	128	112	87.5
20.0×10^{12}	117	92	78.6
22.2×10^{12}	....	....	....
23.0×10^{12}	150	122	81.3
30.0×10^{12}	....	....	....
30.1×10^{12}	....	....	....

[1]All ring configurations were considered to be due to reciprocal interchange. Thus a ring of 6 was counted as 2 interchanges.

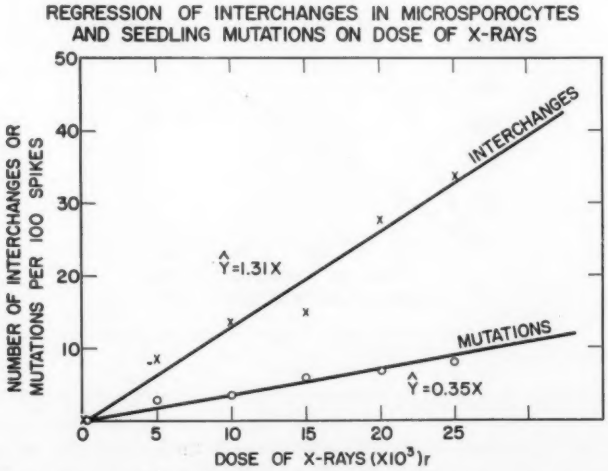

FIGURE 6.—Regression of interchanges in microsporocytes and seedling mutations on dose of X-rays.

that the yield of interchanges induced with X-rays increase at a power greater than unity.

Frequencies of interchanges in X_1 microsporocytes

The frequencies of reciprocal interchanges in microsporocytes of X_1 spikes (X_1 is used to designate the irradiated generation, X_2 the next generation)

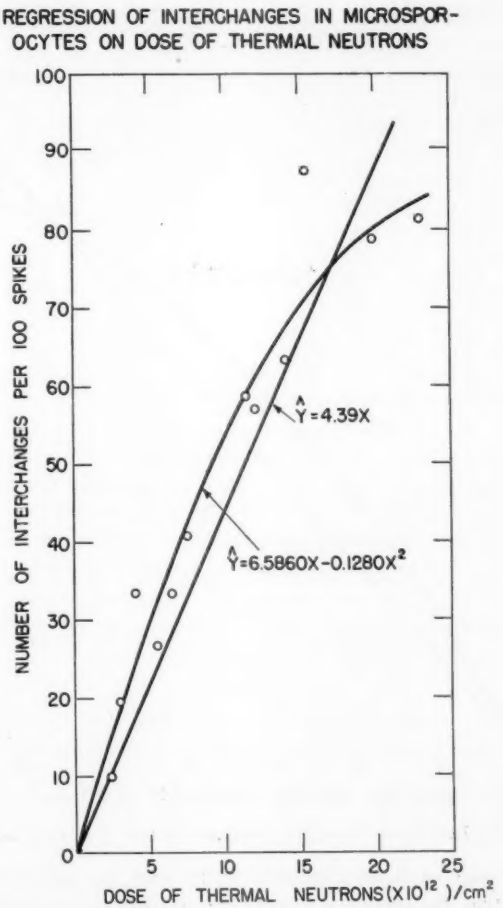

FIGURE 7.—Regression of interchanges in microsporocytes on dose of thermal neutrons.

were determined for all doses of X-rays and thermal neutrons (table 2). The only types of chromosomal anomalies observed at meiosis resulted from reciprocal interchange suggesting that only aberrancies which left the cells essentially genetically whole (exclusive of minute deficiencies) were capable of

persisting to meiosis. No clear case of "gross" chromosome deficiency was observed in the entire study.

In figure 6 the observed interchange frequencies are plotted against dose of X-rays, and the regression line has been drawn to show the relationship that exists. There is no evidence of curvilinearity in these data and 96 percent of the total variation observed in the frequency of interchanges can be accounted

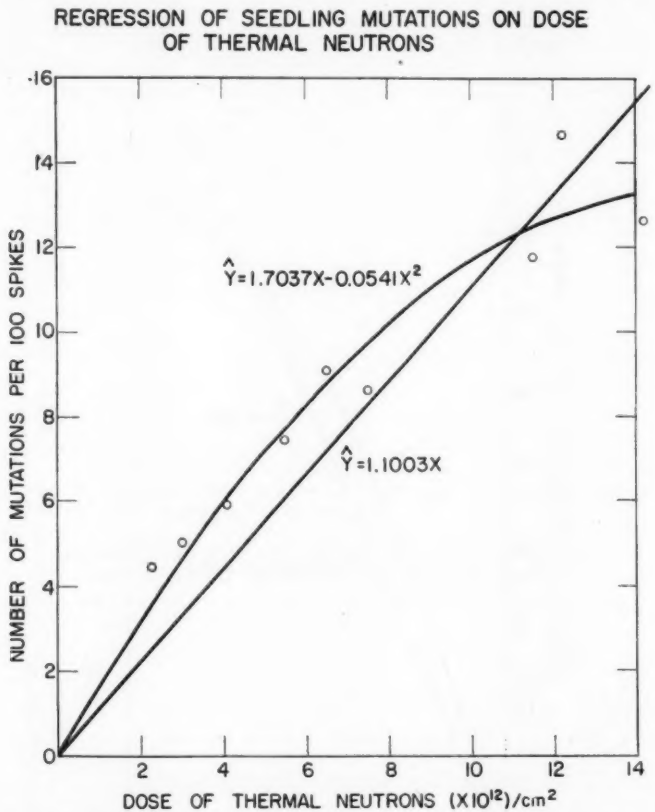

FIGURE 8.—Regression of seedling mutations on dose of thermal neutrons.

for by the dosage of X-rays applied. There were no significant quadratic effects, indicating that there is a straight line relationship between interchange frequency and dose of X-rays.

Preliminary calculations suggested that the relationship between the interchange frequency in microsporocytes and dose of thermal neutrons might be more complex than for X-rays. Therefore, the data were analyzed to determine whether the observed relationship was curvilinear rather than linear. The

TABLE 3

Effect of X-rays and thermal neutrons on dormant seeds of barley as measured by the frequency of seedling mutations.

Treatment	No. spikes tested	Mutations observed	
		Number	Per 100 spikes
Control (no treatment)	1072	1	0.09
Dose of X-rays			
5000 r	4092	119	2.9
10000 r	3055	112	3.7
15000 r	3292	199	6.0
20000 r	2252	153	6.8
25000 r	1331	107	8.0
Dose of thermal neutrons per cm^2			
2.3×10^{12}	3371	151	4.5
3.0×10^{12}	1868	94	5.0
4.1×10^{12}	3036	180	5.9
5.5×10^{12}	2506	187	7.5
6.5×10^{12}	2967	272	9.2
7.5×10^{12}	1019	90	8.8
11.5×10^{12}	1115	132	11.8
12.2×10^{12}	1085	161	14.8
14.2×10^{12}	834	105	12.6

results of this analysis showed a highly significant curvilinear relationship, with the interchange frequency tending to increase less and less with each additional unit dose of thermal neutrons applied. Curvilinear regression can account for 95.3 percent of the total variation in interchange number whereas 86.3 percent can be accounted for by linear regression through the origin. Both regression lines are plotted in figure 7; for their usefulness in interpreting the data, see discussion. It should be pointed out that, although errors in dosimetry are sufficient to be of a serious consequence in a steep curve like

TABLE 4

Frequencies of types of seedling mutants induced in dormant seeds of barley irradiated with X-rays and thermal neutrons.

Mutant types	Treatment				Total	
	X-rays		Thermal neutrons			
	No.	%	No.	%	No.	%
White	387	56.0	779	56.8	1166	56.5
Yellow	102	14.8	140	10.2	242	11.7
Yellow green	114	16.5	237	17.3	351	17.0
Virescent	10	1.4	42	3.1	52	2.5
Striped White and Yellow	17	2.5	36	2.6	53	2.6
Banded Shrivel	20	2.9	52	3.8	72	3.5
Others	41	5.9	86	6.3	127	6.2
Total	691		1372		1063	

χ^2 (6 d.f.) = 14.276*

that for survival, errors of this magnitude would not seriously affect the conclusions about interchanges.

Frequencies of seedling mutations in X_2 progenies

Data on the frequencies of seedling mutations resulting from the different doses of X-radiation and thermal neutron radiation are presented in table 3 and depicted graphically in figures 6 and 8. It is apparent that there is a straight line relationship between mutation frequency and dose of X-rays. For thermal neutrons the relationship was found to fit a curvilinear plot better than a linear plot as was found for the frequency of interchanges. The significance of this latter observation will be dealt with in the discussion.

It will be noted in table 3 (cf. table 1) that mutation data are not presented for the plants which survived the three highest thermal neutron treatments. This is because all tillers on these plants showed a high degree of sterility.

Frequencies of mutant types in X_2 progenies

The types of seedling mutations obtained during the investigation were grouped into the seven classes shown in table 4. A chi square analysis, using a 2×7 contingency table, was conducted to indicate whether or not there was a significant variation in the relative frequencies of the types produced by the two radiations. This chi square value was significant at the 0.05 level. Two of the chi square components (for the mutants yellow and virescent) contributed more than 90 percent to this total value. A chi square test was conducted for these two characters using a 2×2 contingency table. It gave a probability value between 0.30 and 0.20.

Relation of survival to interchange and mutation frequency

The regressions of survival on interchange and mutation frequency are presented in figures 9 and 10 respectively. These relationships demonstrate that much higher chromosomal aberration and mutation frequencies can be obtained for a given survival by irradiating barley seeds with thermal neutrons than can be obtained by irradiating them with X-rays.

Relation of seedling mutations to reciprocal interchange frequencies

Ordinary linear regression of seedling mutations in X_2 progenies on interchange frequencies in pollen mother cells of X_1 spikes was determined for both the X-ray and thermal neutron data. Almost identical regression coefficients (0.185 and 0.177) were obtained in the two analyses. Apparently the coefficients were not dependent on the types of radiation so the data were combined for presentation in figure 11. This relationship indicates that mutations and interchanges are produced in the same relative frequencies when dormant seeds of barley are irradiated with either X-rays or thermal neutrons. It has definite implications relative to the nature of point mutation which will be dealt with in the discussion.

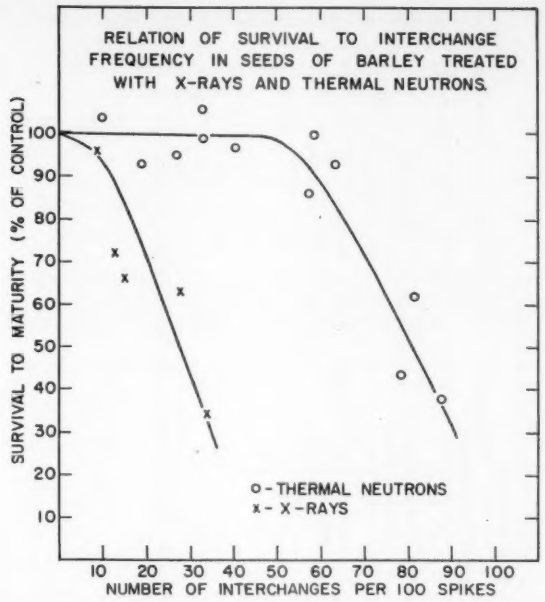

FIGURE 9.—Relation of survival to interchange frequency in seeds of barley treated with X-rays and thermal neutrons.

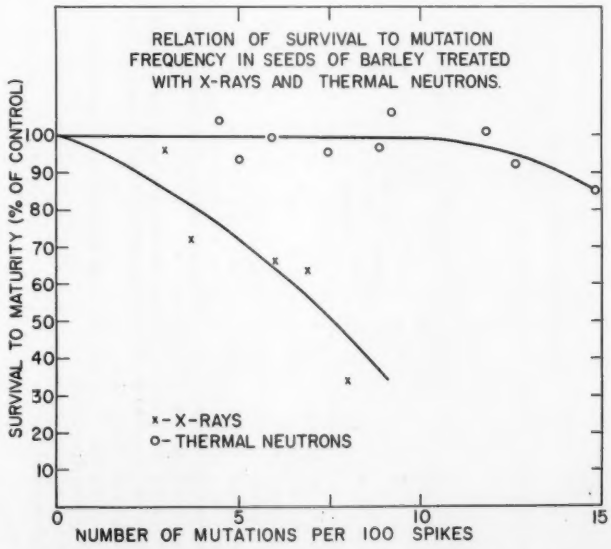

FIGURE 10.—Relation of survival to mutation frequency in seeds of barley treated with X-rays and thermal neutrons.

TABLE 5

Frequencies of interchange types persisting to microsporogenesis from dormant seeds of barley treated with X-rays and thermal neutrons.

Treatment	Spikes analyzed No.	Type of interchange												
		Ring-of-4		Ring-of-6		Two rings-of-4		Two pseudo-iso-chromosomes[1]		Ring-of-4 + Ring-of-6		Ring-of-8		Others[2]
		Total no.	No. per 100 spikes	Total no.	No. per 100 spikes	Total no.	No. per 100 spikes	Total no.	No. per 100 spikes	Total no.	No. per 100 spikes	Total no.	No. per 100 spikes	
Control (No treatment)	173	0	0	0	0	0	0	0	0	0	0	0	0	
X-rays (All doses)	1866	252	13.5	11	0.6	18	1.0	12	0.6	0	0	3	0.2	1
Thermal neutrons (All doses)	1745	486	27.9	67	3.8	52	3.0	21	1.2	10	0.6	12	0.7	9
Combined total	3784	738	19.5	78	2.1	70	1.8	33	0.9	10	0.3	15	0.4	10

[1]Interchanges resulting from exchanges between opposite arms of homologous chromosomes (see Caldecott and Smith, 1953).

[2]The other types were as follows:

A—Ring-of-4 plus two pseudo-iso-chromosomes (three cases).
B—Two Rings-of-4 plus two pseudo-iso-chromosomes (two cases).
C—Three Rings-of-4.
D—Ring-of-4, Ring-of-6 plus two pseudo-iso-chromosomes.
E—Two Rings-of-6.
F—Ring-of-4 plus Ring-of-8.
G—Ring-of-10.

Relation of dosage of X-rays and thermal neutrons to the interchange types obtained

It is important to the cytogeneticist, in his studies of chromosome mechanics, to have data available which pertain to the relation of dosage of a particular radiation to the frequency of the types of monocentric reciprocal interchanges obtained. For this reason the various kinds of interchanges observed during the present study and their relative frequencies are presented in table 5.

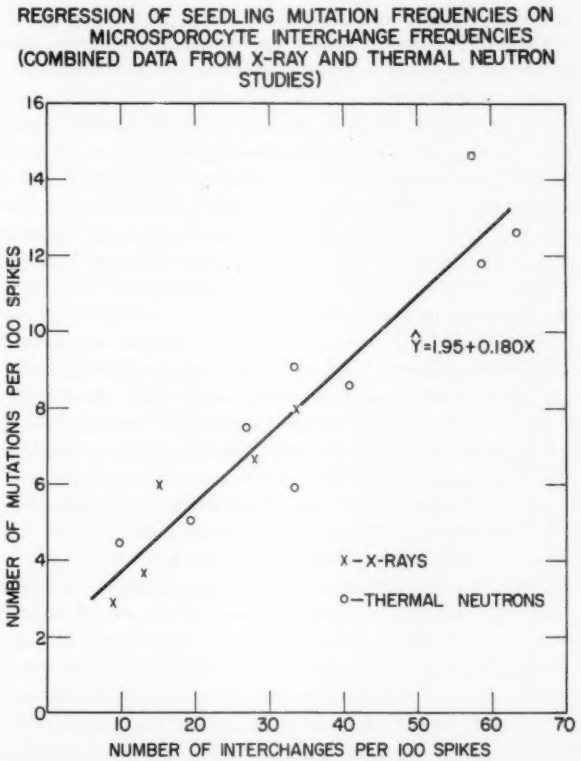

FIGURE 11.—Regression of seedling mutation frequencies on microsporocyte interchange frequencies (combined data from X-ray and thermal neutron studies).

A chi square analysis using a 2×7 contingency table was conducted to determine the likelihood that the two types of radiation produced the same relative frequencies of the different classes of interchanges. The chi square value obtained was significant at the 0.01 level indicating that the two sources of radiation produced different relative frequencies of interchanges. The greatest contribution to the total chi square came from the deviations from expected for rings-of-six and for a ring-of-four plus a ring-of-six. A second chi square

test using a 2×2 contingency table was conducted for these two characters and it was found that the probability of obtaining a larger chi square than that obtained was between 0.05 and 0.10. Further experimentation is needed to establish whether the difference is real or due to chance variation.

DISCUSSION

Most of the biological effect of radiations which induce mutation and chromosomal aberration can be attributed to ionization. It follows that biological events which can be induced by one or a few ionizations should be more efficiently produced by sparsely ionizing radiations and those events which are brought about by dense ion clusters should be most efficiently produced by densely ionizing radiations.

It has been determined by SCHMIDT and FROLIK (1951), that in barley seeds, approximately 98 percent of the ionization produced immediately following thermal neutron capture can be attributed to the emission of protons by nitrogen and alpha particles by boron. These radiations are both densely ionizing and thus for the purposes of this discussion we are essentially dealing with two types of ionizing radiations, viz., (1) densely ionizing radiation from thermal neutron capture and (2) relatively sparsely ionizing electrons produced from absorption of X-ray quanta.

As pointed out earlier, gamma contamination in the thermal column was approximately 50 r per hour. Thus, seeds exposed in the thermal column for the maximum time of 10 hours only received about 500 r. This dose is of little significance in dormant seeds. Therefore, no attempt was made to correct the thermal neutron data for the events attributable to gamma contamination. In the same way the fast neutron contamination was considered to be of relatively minor importance. Furthermore, because fast neutron bombardment primarily results in proton emission it is apparent that this contamination causes dense ion clusters somewhat comparable to those produced by proton and alpha particle emission from thermal neutron capture by nitrogen and boron.

One of the most striking differences between the effects of X-rays and thermal neutrons on dormant seeds of barley is the uniform manner in which a sample of seeds respond to thermal neutron irradiation in comparison with X-radiation (CALDECOTT *et al.* 1952). In the present study this was reflected at all stages of plant development. It is best illustrated by the differences in the variability in seedling heights obtained with the two radiations (figs. 1 and 2). It is also reflected by survival to maturity, when plotted against the dose, where it is apparent that the slope beyond the inflection point is much sharper for thermal neutrons than for X-rays (figs. 3 and 4).

MACKEY (1952) noted that barley seeds are uniformly affected by fast neutrons and ANDERSON *et al.* (1949) and RANDOLPH (1950) have pointed out that maize seeds exposed to the Bikini Test Able Atomic Bomb explosion were much more uniformly affected than seeds exposed to X-rays. These workers data along with those reported herein suggest the possibility that a considerable portion of the biologically effective radiation their seeds received

from the atomic explosion might have been in the form of densely ionizing radiation, particularly neutrons.

The data presented in table 2 and figures 9 and 10 demonstrate that, using survival as a basis for comparison, the frequencies of chromosomal interchanges obtained with thermal neutrons were far in excess of those obtained with X-radiation. From this it seems that the killing effect of X-rays cannot be attributed to chromosomal anomalies of the types reported herein. Whether or not X-rays may affect the chromosomes in a manner not detected in the analyses is a question. However, if one assumes that this is unlikely, one is inclined to favor the explanation previously proposed by CALDECOTT *et al.* (1952), viz., that X-rays have a pronounced effect on extra-chromosomal constituents of the cell. This would suggest that chromosome breaks must require a considerable number of ionizations in a very limited area whereas at least some of the nongenetic injury resulting in killing apparently results from a more uniform distribution of ion pairs throughout the entire system.

MACKEY (1951, 1952) compared the effect of fast neutrons and X-rays on seeds of barley and presented data very similar to those presented herein. He concluded that X-rays must have a pronounced physiological effect on biological systems as did SAX and SWANSON (1941).

As with the frequency of chromosomal aberrations, at equivalent survival values, much higher mutation frequencies were obtained in the material treated with thermal neutrons than in the material treated with X-rays. The data indicate that the immediate lethal effect of X-rays is not due to the induction of deleterious mutations and it appears that other factors must be involved.

The regression lines in figures 7 and 8 indicate that interchanges in pollen mother cells and seedling mutations may not increase in a strictly linear fashion with the dose of thermal neutrons but may tend to fall off at higher doses. This is very likely a reflection of the fact that cells which are most severely "genetically injured" are unable to survive or compete with less seriously damaged cells during the ontogeny of the plant. Further evidence in this connection is the fact that interchanges in the first cycle of cell divisions, where there has been no genetic selection for survival (see fig. 5), increase linearly with the dose. That a similar relation of aberration and mutation to dose is not reflected in the X-rayed material probably is due to the fact that relatively fewer cells are sufficiently damaged "genetically" by X-radiation to cause cell death. This is evidence that factors leading to nongenetic injury or death of the seedling or plant, such as described above for X-radiation, do not selectively kill genetically damaged as compared to genetically undamaged cells over the dosage range of X-rays used.

SAX (1941), working with the microspore chromosomes of Tradescantia, and a number of other workers have shown that the yield of two break aberrations induced with X-radiation increases as the square of the dose or at least at a power greater than unity. This presumably results from the fact that the probability of two breaks being "simultaneously" present in a position to permit exchange increases with the dose of X-rays. On the other hand,

it has been shown that the frequency of two break aberrations increases linearly with the dose of densely ionizing, particulate radiations (GILES 1940, 1943). This is given as evidence that the dense ionization track resulting from this type of radiation usually causes two breaks which are " simultaneously " present and have a good probability of exchange.

From figures 5, 6 and 7 it seems apparent that in dormant seeds there is actually a linear relationship between interchange frequency and dose of both X-rays and thermal neutrons. This suggests that the two radiations may be inducing exchanges in a similar fashion. It should be emphasized that the linear relation of interchange frequency to dosage of X-rays was obtained by LUTHER SMITH and the senior author (unpublished results) over a range of doses from 0 to 32,000 r and was observed in four separate experiments. We are tentatively working on the hypothesis that the " simultaneous " production of two breaks, needed to explain the linear relationship between dose of X-rays and interchange frequency, result from dense ionization at the end of an electron track (dubbed electron " tail " by LEA 1947).

Since it was first shown that ionizing radiations can profoundly affect the rate of mutation and the frequency of structural alterations in chromosomes, geneticists have been seeking some way to control and direct these changes. In this connection, it was hoped that thermal neutrons would produce a different mutation pattern than X-rays thus helping to elucidate this very complex problem.

On purely physical considerations this did not seem unreasonable because the capture cross section of some atoms for thermal neutrons is much higher than for others (e.g., nitrogen and boron as compared with carbon). Thus, one might expect certain loci in the chromosomes to be ionized more frequently than other loci. Because there was no obvious reason to suspect that the loci most affected by thermal neutrons would be the ones most frequently ionized by the relatively unselective X-rays, it was felt that there was reason to hope the mutation types induced by the two radiations might be particularly revealing. It was also felt that if a gene mutated by virtue of its chemical properties being altered, thermal neutron capture might cause many point mutations due to transmutation. In this connection, the data presented in table 4 show no striking differences in the types of mutations induced by the two radiations, although they suggest that there may be a difference.

It should be pointed out that the mutant classes recorded in table 4 are each made up of two or more types. Thus, in the strict sense they do not indicate a change in a specific gene but rather in a class of genes which produce somewhat comparable phenotypes. This method of classification must mask small differences in the mutant types produced by the two radiations. It is not deemed advisable to attempt to further separate the types because both temperature and illumination are known to exert considerable influence on the degree of pigmentation and leaf development and variations in these two factors might confuse the issue.

A comparison of the relation of mutation to chromosomal exchange for the

two types of radiation was particularly interesting (fig. 11). It indicated that, per interchange, about the same number of mutations is induced by both X-rays and thermal neutrons. If the mutations observed were induced by one or a few ionizations one would have expected X-radiation to incite more mutations relative to aberrations than thermal neutrons. As this is not the case it appears that true point mutations were not observed but rather phenotypes which result from structural anomalies (e.g., deficiency and/or position effect). This suggests that true point mutations may be only rarely, if at all, produced in dormant seeds of barley by ionizing radiations and supports the findings of STADLER and ROMAN (1943, 1948).

The biological equivalent of thermal neutrons to X-rays in producing chromosomal aberrations was calculated to be approximately 3.0×10^8 thermal neutrons per cm^2 equal 1 r. Essentially, the same equivalence figure was obtained for the two radiations in producing mutations.

It is of interest that, as based on the production of chromosomal aberrations in Tradescantia microsporocytes, CONGER and GILES (1950) calculated that approximately 5×10^9 thermal neutrons per cm^2 were equal to 1 r of X-rays. This indicates that thermal neutrons are approximately 16 times more efficient at breaking chromosomes in dormant seeds of barley than they are in Tradescantia microspores.

It is suggested as a possibility that a portion of the difference in the equivalence values obtained by CONGER and GILES and in the present study may result from the production and transport of active radicals (e.g., H, OH, HO_2) produced by X-radiation. It appears reasonable to assume that in tissues with a high water content and metabolic rate, such as Tradescantia microspores, these radicals would be more readily produced and transported (e.g., within the nucleus or from cytoplasm to nucleus) than in relatively dry dormant seeds. A small part of the difference in equivalence values may be due to the fact that CONGER and GILES could only attribute 77 percent of the total ionization dose to proton and alpha rays whereas in this study over 90 percent could be attributed to these two radiations.

SUMMARY

A comparison is made between the effects of irradiating dormant seeds of barley with X-rays and thermal neutrons as measured by survival and the frequency of chromosomal aberrations and mutations. The following observations and conclusions resulted from the investigation.

1. Over the range of doses used, seeds subjected to thermal neutron irradiation were much more uniformly affected by the irradiation than seeds subjected to X-radiation. This is reflected by the variability in seedling heights obtained with X-radiation in comparison with thermal neutron irradiation (figs. 1–2). It is also evidenced by survival to maturity where it is apparent that, when survival is plotted against the dose, the slope beyond the inflection point is much sharper for thermal neutrons than for X-rays (figs. 3–4).

2. The frequencies of chromosomal bridges in root tip cells and interchanges

in pollen mother cells obtained with the highest non-lethal dose of thermal neutrons was about 2.5 times the value obtained with 20,000 r of X-rays.

3. In the same way, the highest mutation frequency obtained with thermal neutron treatments, which did not cause a high degree of sterility, was about 2 times that obtained with 20,000 r of X-rays.

4. Because thermal neutrons may induce more chromosomal aberrations and mutations but cause less killing than X-rays, it was suggested that X-rays must have a pronounced effect on extra-chromosomal constituents of the cell and probably cause killing due to nongenetic injury. It is also considered possible that X-rays may affect the chromosomes in a cytologically undetectable manner.

5. The data suggest that there may be a difference in the types and frequencies of monocentric reciprocal interchanges and seedling mutations induced by the two radiations.

6. Essentially, there was a straight line relationship between dose of X-rays and thermal neutrons and the frequency of induced mutations and chromosomal interchanges.

7. Per interchange in pollen mother cells, as many mutations were induced with X-rays as with thermal neutrons.

8. Apparently, most of the ionization produced in the seeds by thermal neutron capture was caused by protons from nitrogen and alpha particles from boron. As these are densely ionizing radiations, it was concluded that both the seedling mutations and chromosomal aberrations observed resulted from the production of numerous ion pairs at a locus. It may be evidence that most of the seedling mutations observed resulted from deficiency and/or position effect.

9. In terms of the production of both chromosomal aberrations and mutations it was calculated that approximately 3.0×10^8 thermal neutrons per cm^2 were equivalent to 1 r from X-radiation.

10. It was suggested that this high equivalence value could possibly be attributed to the fact that active radicals resulting from X-radiation may be intracellularly produced and transported in a very reduced frequency in dormant seeds as compared with relatively moist actively metabolizing tissue, e.g., the Tradescantia microspores of CONGER and GILES (1950).

ACKNOWLEDGMENT

The writers are indebted to DR. E. F. FROLIK, Chairman of the Department of Agronomy, at the University of Nebraska for the excellent cooperation provided during the investigation. They are also indebted to DR. ROSALIND MORRIS, N. A. WILLIAMS, P. YAGYU, MILDRED R. BROWN and S. PERSON for assisting with various phases of the work.

Special thanks are tendered to DR. F. FORRO, and other members of the Biology Department at the Brookhaven National Laboratory, for many constructive suggestions during the course of the work and preparation of the manuscript.

LITERATURE CITED

ANDERSON, E. G., A. E. LONGLEY, C. H. LI and K. L. RETHERFORD, 1949 Hereditary effects produced in maize by radiations from the Bikini Atom Bomb. 1. Studies on seedlings and pollen of the exposed generation. Genetics **34:** 639–646.

CALDECOTT, R. S., and L. SMITH, 1952a The influence of heat treatment on the injury and cytogenetic effects of X-rays on barley. Genetics **37:** 136–157.

1952b A study of x-ray-induced chromosomal aberrations in barley. Cytologia **17:** 224–242.

CALDECOTT, R. S., E. F. FROLIK and ROSALIND MORRIS, 1952 A comparison of the effects of x-rays and thermal neutrons on dormant seeds of barley. Proc. Nat. Acad. Sci. **38:** 804–809.

CONGER, A. D., and N. H. GILES, 1950 The cytogenetic effect of slow neutrons. Genetics **35:** 397–419.

GILES, N. H., 1940 Induction of chromosome aberrations by neutrons in Tradescantia microspores. Proc. Nat. Acad. Sci. **26:** 567–575.

1943 Comparative studies of the cytogenetic effects of neutrons and X-rays. Genetics **28:** 398–418.

LEA, D. E., 1947 Actions of radiations on living cells. New York. The MacMillan Co.

MACKEY, J., 1951 Neutron and x-ray experiments in barley. Hereditas **37:** 421–464.

1952 The biological action of x-rays and fast neutrons on barley and wheat. Arkiv för Botanik. **1**(16) : 545–556.

RANDOLPH, L. F., 1950 Cytological and phenotypical effects induced in maize by x-rays and the Bikini Test Able Atomic Bomb. Jour. Cell. and Comp. Physiol. (supplement 1) **35:** 103–118.

SAX, K., 1941 Types and frequencies of chromosomal aberrations induced by x-rays. Cold Spring Harbor Symp. Quant. Biol. **9:** 93–101.

SAX, K., and C. P. SWANSON, 1941 Differential sensitivity of cells to x-rays. Amer. J. Bot. **28:** 52–59.

SCHMIDT, J. W., and E. F. FROLIK, 1951 The effects of thermal neutron irradiation of maize and barley kernels. University of Nebraska Research Bulletin 167, 29 pp.

SPARROW, A. H., and B. A. RUBIN, 1952 Effects of radiations on biological systems. Survey of Biological Progress **2:** 1–52.

STADLER, L. J., 1930 Some genetic effects of x-rays in plants. J. Hered. **21:** 3–19.

STADLER, L. J., and H. ROMAN, 1943 The genetic nature of X-ray and ultraviolet induced mutations affecting the gene A in maize. Genetics **28:** 91.

1948 The effect of X-rays upon mutation of the gene A in maize. Genetics **33:** 273–303.

JOURNAL OF GENETICS

Editor: J. B. S. HALDANE

FOUNDED in 1910 the journal publishes records of original research in Heredity Variation and allied subjects, and also from time to time articles summarizing the existing state of knowledge in the various branches of Genetics. All articles are illustrated both with text-figures, and plates, the latter in black and white, and colors.

Subscription price, per volume of 3 parts is $12.00 net, post free. Single parts $5.00 net, postage extra. Quotations for back numbers on application.

Orders to

CAMBRIDGE UNIVERSITY PRESS
32 East 57th Street,
New York 22, N. Y.

ANNALS OF EUGENICS

A JOURNAL OF HUMAN GENETICS

Edited by
L. S. PENROSE
with the assistance of

JULIA BELL • R. A. FISHER
J. B. S. HALDANE • R. R. RACE
M. N. KARN • J. A. F. ROBERTS

Subscription in advance
$9.50 per volume of 4 parts

Orders should be placed with

CAMBRIDGE UNIVERSITY PRESS
32 East 57th Street, New York 22

ECOLOGICAL MONOGRAPHS

(Official Publication of the Ecological Society of America)

First issue published January, 1931

Managing Editors: H. J. OOSTING, *Duke University* and WILLIAM J. HAMILTON, JR., *Cornell University*

A quarterly journal devoted to the publication of original researches of ecological interest from the entire field of biological science. The journal will work in close cooperation with *Ecology* and will undertake the publication of papers of from 25 to 100 printed pages in length while *Ecology* will continue to specialize on papers of about 20 printed pages or less.

Published in January, April, July, and October

SUBSCRIPTION: $6.00

Back volumes as available, $7.00.
Postage extra on quantity shipments.

DUKE UNIVERSITY PRESS
COLLEGE STATION, BOX 6697
DURHAM, NORTH CAROLINA

Subscribe to Wistar Institute scientific periodicals for authentic research literature in the biological sciences and nutrition.

JOURNAL OF MORPHOLOGY
$20.00 domestic, $21.00 foreign

THE JOURNAL OF COMPARATIVE NEUROLOGY
$15.00 domestic, $16.00 foreign

THE AMERICAN JOURNAL OF ANATOMY
$15.00 domestic, $16.00 foreign

THE ANATOMICAL RECORD
$22.50 domestic, $24.00 foreign

THE JOURNAL OF EXPERIMENTAL ZOOLOGY
$22.50 domestic, $24.00 foreign

AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY
$7.50 domestic, $8.00 foreign

JOURNAL OF CELLULAR AND COMPARATIVE PHYSIOLOGY
$15.00 domestic, $16.00 foreign

THE JOURNAL OF NUTRITION
$22.50 domestic, $24.00 foreign

Send orders with remittance to

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
36th Street and Woodland Avenue
Philadelphia 4, Pa.

BIOLOGICAL ABSTRACTS

Covers the world's biological literature

How do you keep abreast of the literature in your field? Perhaps some relatively obscure journal has published a revealing paper on the very subject in which you are most interested. Informative, concise abridgments of all the significant contributions will be found in *Biological Abstracts.*

As well as the complete edition, covering all fields of biology, *Biological Abstracts* also is published in five low-priced sectional editions which are specially designed for individuals who are interested only in one or more closely related fields. Section A affords a very complete coverage of the Genetics literature—and it is priced at only $5.00 a year (Foreign $5.50).

Write for a sample copy.

BIOLOGICAL ABSTRACTS

University of Pennsylvania
Philadelphia 4, Pa.

GENETICS SOCIETY OF AMERICA

EXECUTIVE COMMITTEE

President, J. T. PATTERSON — *Vice-President*, R. A. BRINK

Past Presidents, J. W. GOWEN, ROY E. CLAUSEN

Secretary, C. P. OLIVER
University of Texas
Austin 12, Texas

Treasurer, N. H. GILES, JR.
Yale University
New Haven, Connecticut

"The Genetics Society of America is organized to provide facilities for association and conference among students of heredity and for the encouragement of close relationship between workers in genetics and those in related subjects. All persons actively interested in any field of genetics shall be eligible to active membership. Candidates for membership must be recommended by two members of the Society. Members shall be elected by the Executive Committee."—Constitution, Article I.

Any one interested in joining the Society should write to the Secretary.

The Genetics Society publishes annually the Records of the Society containing the program and abstracts of the Annual Meeting and others items of interest to members, including a membership list at periodic intervals. Total membership of the Society was 950 on September 10, 1952. Copies of the Records may be obtained from the Treasurer for $1.00. The Genetics Society has a representative on the Editorial Board of GENETICS, Tracy M. Sonneborn.

SUSTAINING MEMBERS

The Genetics Society of America established in 1949 a new type of membership, Sustaining Members. The constitution provides that "All organizations interested in any field of genetics shall be eligible to sustaining membership." The dues for sustaining members are $50.00 a year. Money contributed by sustaining members is not used for running expenses of the Society, but is used for special purposes as decided by the Executive Committee. The Executive Committee at a meeting in Ithaca, New York on December 29, 1953, voted unanimously that $1,000 from the sustaining membership account be allocated to GENETICS toward its publication costs.

The following organizations became sustaining members of the Society in the years indicated:

Associated Seed Growers
New Haven, Conn.—1950

Bear Hybrids Corn Co.
Decatur, Ill.—1950

W. Atlee Burpee Co.
Philadelphia 32, Pa.—1950

DeKalb Hybrid Seed Co.
DeKalb, Ill.—1949

Ferry-Morse Seed Co.
Detroit 31, Mich.—1949

Funk Brothers Seed Co.
Bloomington, Ill.—1950

Kimber Farms
Niles, California—1953

Moews Seed Co.
Granville, Ill.—1949

Nichols Poultry Farm
Kingston, N. H.—1950

Pioneer Hi-Bred Corn Co.
Johnston, Iowa—1949

This page is donated by GENETICS to the GENETICS SOCIETY OF AMERICA. The society and the journal are separate organizations with a common bond of interest in furthering the science of heredity.

INFORMATION FOR CONTRIBUTORS

Contributions to GENETICS may be in the field of genetics proper, or in any scientific field, if of such a character as to be of primary genetic interest.

The length of manuscripts will be limited to twenty printed pages, including tables and illustrations. Longer papers may be published by special vote of the Editorial Board and if the author agrees to bear the cost of the additional pages. In case unusually extensive tabular material cannot be printed, it will be kept on file on request, provided two copies are furnished by the author.

The *Galton and Mendel Memorial Fund* established in 1923 from donations of biologists and other persons interested in the progress of biological discovery is available to be applied toward the cost of reproducing illustrations and of printing expensive tables and formulae.

Authors should submit two copies of their manuscripts to the Editors. Manuscripts should conform to the general usage in GENETICS, particularly in regard to references to literature, arrangement of "Literature Cited" and inclusion of a summary. In case of "Acknowledgments," these should follow the "Summary." Footnotes should be avoided in the text whenever possible and their content be inserted, in parentheses, after the appropriate sentence. Footnotes to tables should be marked with an asterisk, dagger, or other symbol so as not to be confused with the figures in tables. Legends for figures and plates should be type-written separately from the illustrations. In addition to the original illustrations photographic copies should accompany the manuscript. Tables also should be type-written separately. The metric system should be used whenever possible.

Galley proofs and page proofs will be sent to authors, except in case of authors resident overseas who, in general, will receive galley proofs only. Authors should leave forwarding directions whenever they are to be away from the address sent with the manuscript.

Reprints may be ordered at the time galley proof is returned. The author will be billed for them in accordance with the schedule of charges appearing on the order blank. Copies of the schedule may be obtained from the Editorial Office.

Manuscripts and editorial correspondence should be addressed to the Editors of GENETICS, Genetics Building, University of Wisconsin, Madison 6, Wisconsin.

GENETICS Vol. 39 No. 2 MARCH, 1954

CONTENTS OF PRECEDING ISSUES

JULY, 1953

HOUSE, VERL L., The interaction of mutants affecting venation in *Drosophila melanogaster*. II. Additive and pattern effects of combinations of Hairless, engrailed and cubitus interruptus.

BAKER, WILLIAM K., V-type position effects of a gene in *Drosophila virilis* normally located in heterochromatin.

LEFEVRE, G., JR., F. J. RATTY, JR. and G. D. HANKS, Frequency of Notch mutations induced in normal, duplicated and inverted X-chromosomes of *Drosophila melanogaster*.

HOROWITZ, N. H., and MARGUERITE FLING, Genetic determination of tyrosinase thermostability in Neurospora.

KIRBY-SMITH, J. S., and D. S. DANIELS, The relative effects of X-rays, gamma rays and beta rays on chromosomal breakage in Tradescantia.

STINSON, HARRY T., Cytogenetics and phylogeny of *Oenothera argillicola* Mackenz.

DE SERRES, FREDERICK J., and NORMAN H. GILES, The effect of radiation dose fractionation on chromosome aberration frequencies in Tradescantia microspores. I. Studies with X-rays.

GILES, NORMAN H., FREDERICK J. DE SERRES and ALVIN V. BEATTY, The effect of radiation dose fractionation on chromosome aberration frequencies in Tradescantia microspores. II. Studies with fast neutrons.

SEPTEMBER, 1953

KYLE, W. H., and A. B. CHAPMAN, Experimental check of the effectiveness of selection for a quantitative character.

BREWBAKER, JAMES L., Oppositional allelism in diploid and autotetraploid *Trifolium hybridum* L.

WALLACE, BRUCE, and CAROL MADDEN, The frequencies of sub- and supervitals in experimental populations of *Drosophila melanogaster*.

DOBZHANSKY, THEODOSIUS, and BORIS SPASSKY, Genetics of natural populations. XXI. Concealed variability in two sympatric species of Drosophila.

LAUGHNAN, JOHN R., The effect of the *sh*$_2$ factor on carbohydrate reserves in the mature endosperm of maize.

LEVINTHAL, C., and N. VISCONTI, Growth and recombination in bacterial viruses.

DUNN, L. C., and S. GLUECKSOHN-WAELSCH, The failure of a t-allele (t^8) to suppress crossing over in the mouse.

PROUT, TIMOTHY, CHARLES HUEBSCHMAN, HOWARD LEVENE and FRANCIS J. RYAN, The proportions of nuclear types in Neurospora heterocaryons as determined by plating conidia.

NOVEMBER, 1953

GOWEN, JOHN W., JANICE STADLER, H. H. PLOUGH and HELEN N. MILLER, Virulence and immunizing capacity of *Salmonella typhimurium* as related to mutations in metabolic requirements.

SIEGEL, R. W., A genetic analysis of the mate-killer trait in *Paramecium aurelia*, variety 8.

LEVINE, MYRON, The diverse mate-killers of *Paramecium aurelia*, variety 8: their interrelations and genetic basis.

MCCLINTOCK, BARBARA, Induction of instability at selected loci in maize.

STEBBINS, G. L., and FUNG TING PUN, Artificial and natural hybrids in the Gramineae, tribe Hordeae. VI. Chromosome pairing in *Secale cereale* × *Agropyron intermedium* and the problem of genome homologies in the Triticinae.

EATON, ORSON N., Heterosis in the performance of mice.

KAPLAN, WILLIAM D., The influence of Minutes upon somatic crossing over in *Drosophila melanogaster*.

Abstracts of papers presented at the 1953 Meetings of the Genetics Society of America.

JANUARY, 1954

PLOUGH, HAROLD H., Edwin Grant Conklin.

STALKER, HARRISON D., Parthenogenesis in Drosophila.

CASTLE, W. E., Coat color inheritance in horses and in other mammals.

SLATIS, HERMAN M., and DAVID A. WILLEMET, The miniature complex in *Drosophila melanogaster*.

SWAMINATHAN, M. S., Nature of polyploidy in some 48-chromosome species of the genus Solanum, section Tuberarium.

BRNCIC, DANKO, Heterosis and the integration of the genotype in geographic populations of *Drosophila pseudoobscura*.

CAIN, A. J., and P. M. SHEPPARD, Natural selection in Cepaea.

AVERS, CHARLOTTE J., Chromosome behavior in fertile triploid Aster hybrids.

NOVITSKI, EDWARD, The compound X chromosomes in Drosophila.

SINGLE NUMBERS $1.50 ANNUAL SUBSCRIPTION $8.00

obtainable from

GENETICS, INC., THE SCIENCE PRESS, BOX 749, LANCASTER, PA.